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# CLINICAL HEMATOLOGY

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**Gamal Abdul Hamid, MD,PhD**

# **CLINICAL HEMATOLOGY**

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## **PREFACE**

Clinical Hematology, first edition is written specifically for medical students, the clinician and resident doctors in training and general practitioner. It is a practical guide to the diagnosis and treatment of the most common disorders of red blood cells, white blood cells, hemostasis and blood transfusion medicine.

Each disease state is discussed in terms of the pathophysiology, clinical and paraclinical features which support the diagnosis and differential diagnosis. We bring together facts, concepts, and protocols important for the practice of hematology. In addition this book is also supported with review questions and quizzes.

I would like to thank Prof Dr Anisa Abood for reviewing the typescript and for some advises may help undergraduate students in this field.

G.A-H

2013

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# HEMATOPOIESIS

## 1

All of the cells in the peripheral blood have finite life spans and thus must be renewed continuously. The mechanisms responsible for regulating steady-state hematopoiesis and the capacity to modulate blood cell production in response to stresses such as anemia or infection consist of a series of progenitor cells in the bone marrow and a complex array of regulatory factors.

It is the process of blood cell production, differentiation, and development. The hematopoietic system consists of the bone marrow, liver, spleen, lymphnodes, and thymus.

It starts as early as the 3<sup>rd</sup> week of gestation in the yolk sac. By the 2<sup>nd</sup> month, hematopoiesis is established in the liver and continuous through the 2<sup>nd</sup> trimester.

During the 3<sup>rd</sup> trimester it shifts gradually to bone marrow cavities. During infancy: all marrow cavities are active in erythropoiesis "Red Marrow". During childhood: erythropoiesis becomes gradually restricted to flat bones as; skull, vertebrae, sternum, Ribs and pelvic bones, in addition to ends of long bones. The shafts of long bones become populated by fat "yellow marrow".

### Blood Cell Development

The pluripotent stem cell is the first in a sequence of steps of hematopoietic cell generation and maturation. The progenitor of all blood cells is called the multipotential hematopoietic stem cell. These cells have the capacity for self-renewal as well as proliferation and differentiation into progenitor cells committed to one specific cell line.

The multipotential stem cell is the progenitor for two major ancestral cell lines: Lymphocytic and non-lymphocytic cells. The lymphoid stem cell is the precursor of mature T cells or B cells/ plasma cells. The non-lymphocytic (myeloid) stem cell is progress to the progenitor CFU-GEMM (colony-forming unit granulocyte-erythrocyte-monocyte-megakaryocyte). The CFU-GEMM can lead to the formation of CFU-GM (CFU-granulocyte-macrophage / monocyte), CFU-Eo (CF-Eosinophil), CFU-Bs (CFU-basophil) And CFU-MEG (CFU-Megakaryocyte). In erythropoiesis, the CFU-GEMM differentiates, into the BFU-E (Burst-Forming unit Erythroid). Each of the CFUs in turn can produce a colony of one hematopoietic lineage under appropriate growth conditions. CFU-E is the target cells for erythropoietin.

### Hematopoietic Growth Factors

The hematopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of hematopoietic progenitor cells and the function of mature blood cells. These growth factors were referred to as colony stimulating factors (CSFs) because they stimulated the formation of colonies of cells derived from individual bone marrow progenitors. Erythropoietin, granulocyte-macrophage colony stimulating factors (GM-CSF) granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF) and interleukin-3 are representative factor that have been identified, cloned and produced through recombinant DNA technology.

The hematopoietic growth factors interact with blood cells at different levels in the cascade of cell differentiation from the multipotential progenitor to the circulating mature cell.



**Table 1.1: Human hematopoietic growth factors**

Growth Factor	Source	Major Function
GM-CSF	T-Lymphocyte, endothelial cells, Fibroblasts	Stimulates production of neutrophils, eosinophils, monocytes, red cells and platelets.
G-CSF	Monocytes, Fibroblasts	Stimulates production of neutrophils.
M-CSF	Macrophages, endothelial cells	Stimulates production of monocytes
ERYTHROPOIETIN	Peritubular cells, Liver, Macrophages	Stimulates production of red cells.
IL-1	Macrophages, activated lymphocytes, endothelial cells.	Cofactor for IL-3 and IL-6. Activated T cells
IL-2	Activated T cells	T cell growth factor. Stimulates IL-1 synthesis. Activated B cells and NK cells
IL-3	T cells	Stimulates production of all non-lymphoid cells.
IL-4	Activated T cells	Growth factor for activated B cells, resting T cells and mast cells.
IL-5	T cells	Induces differentiation of activated B cells and eosinophils.
IL-6	T cells	Stimulates CFU-GEMM Stimulates Ig synthesis
IL-7	T cells, Fibroblasts, Endothelial cells	Growth factor for pre B cells

### Development and Maturation

**Erythrocytes** are rapidly maturing cells that undergo several mitotic divisions during the maturation process. The Pronormoblast" is the first identifiable cell of this line followed by the " Basophilic normoblast ", polychromatic normoblast ", orthochromatic normocyte " and reticulocyte stages in the bone marrow. Reticulocytes enter the circulating blood and fully mature into functioned erythrocytes.

A defect in nuclear maturation can occur. This is referred to as megaloblastic maturation. In this condition, the nuclear maturation, which represents an impaired ability of the cell to synthesize DNA, lags behind the normally developing cytoplasm.

Reticulocytes represent the first nonnucleated stage in erythrocytic development. Although the nucleus has been lost from the cell by this stage, as long as RNA is present, synthesis of both protein and heme continues. The ultimate catabolism of RNA, ribosome disintegration, and loss of mitochondria mark the transition from the reticulocyte stage to full maturation of the erythrocyte. If erythropoietin stimulation produces increased numbers of immature reticulocytes in the blood circulation, these Reticulocytes are referred to as stress or shift reticulocytes. Supravital stains such as new methylene blue are used to perform quantitative determination of blood reticulocytes.

**Figure 1.1: Developmental characteristics of erythrocytes**

**Pronormoblast**

Size 12- 19  $\mu\text{m}$  in diameter  
 N:C ratio 4:1  
 Nucleus Large, round nucleus  
 Chromatin has a fine pattern 0-2 nucleoli  
 Cytoplasm: distinctive basophilic colour without granules

**Basophilic Normoblast**

Size 12- 17  $\mu\text{m}$  in diameter  
 N:C ratio 4: 1  
 Nucleus:  
 Nuclear chromatin more clumped  
 Nucleoli usually not apparent  
 Cytoplasm: Distinctive basophilic colour

**Polychromatic Normoblast**

Size 11-1  $\mu\text{m}$  in diameter  
 N:C ratio 1:1  
 Nucleus:  
 Increased clumping of the chromatin  
 Cytoplasm: Colour: Variable, with pink staining  
 Mixed with Basophilia

**Orthochromic Normoblast or nucleated RBC**

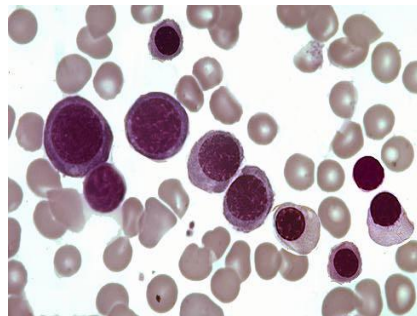
Size: 85-12  $\mu\text{m}$   
 Nucleus:  
 Chromatin pattern is tightly condensed.  
 Cytoplasm:  
 Colour: reddish-pink (acidophilic)

**Reticulocyte (Supravital stain)**

Size 7-10  $\mu\text{m}$   
 Cell is anuclear  
 Polychromatic Erythrocyte  
 Diffuse reticulum (Wright stain)  
 Cytoplasm:  
 Overall blue appearance

**Erythrocyte**

Average diameter 6-8  $\mu\text{m}$



Pronormoblast (1) → basophilic normoblast (2) → polychromatic N (3-4) → Orthochromic normoblast (5-6)

The **myeloblast** is the first identifiable cell in the granulocytic series. Myeloblast constitutes approximately 1% of the total nucleated bone marrow cells. This stage lasts about 15 hours. The next stage, the promyelocyte, constitutes approximately 3% of the nucleated bone marrow; this stage lasts about 24 hours. The myelocyte is the next maturational stage, with approximately 12% of the proliferative cells existing in this stage. The stage from myelocyte to metamyelocyte lasts an average 4.3 days. The time required for the division and maturation of a myeloblast to a mature granulocyte is 5-12 days. Two stages of granulocytes are observed in the circulating blood: the band form of neutrophils, eosinophils and basophils and in end stage of maturation.

The normal number of neutrophilic granulocytes in the peripheral blood is about 2500-7500/ $\mu$ l. Neutrophilic granulocytes have a dense nucleus split into two to five lobes and a pale cytoplasm. The cytoplasm contains numerous pink blue or gray blue granules. Two types of granules can be distinguished morphologically; primary or azurophilic granules which appear at the promyelocyte stage and secondary granules, which appear later. The primary granules contain myeloperoxidase, and acid hydrolase, whereas lyszymes, lactoferrin, and collagenase are found in the secondary granules.

It has been estimated that  $1.5 \times 10^9$  granulocytes/kg are produced daily in the healthy organism. Most of these cells stay at various stages of maturation in the bone marrow from where they can be mobilized in case of lymphopoietic stress. Following their release from the bone marrow, granulocytes circulate for no longer than 12 h in the blood. About half of the granulocytes present in the blood stream are found in the circulating pool, whereas the other half is kept in a marginated pool attached to blood vessel walls. After granulocytes move from the circulation into tissues, they survive for about 5 days before they die while fighting infection or as a result of senescence.

The major function of granulocytes (neutrophils) is the uptake and killing of bacterial pathogens. The first step involves the process of chemotaxis by which the granulocyte is attracted to the pathogen. Chemotaxis is initiated by chemotactic factors released from damaged tissues or complement components. The next step is phagocytosis or the actual ingestion of the bacteria, fungi, or other particles by the granulocyte. The recognition and uptake of a foreign particle is made easier if the particle is opsonized. This is done by coating them with antibody or complement. The coated particles then bind to Fc or C3b receptors on the granulocytes. Opsonization is also involved in the phagocytosis of bacteria or other pathogens by monocytes. During phagocytosis a vesicle is formed in the phagocytic cell into which enzymes are released. These enzymes, including collagenase, amino peptidase, and lysozyme, derive from the secondary granules of the granulocyte.

The final step in the phagocytic process is the killing and digestion of the pathogen. This is achieved by both oxygen dependent and independent pathways. In the oxygen-dependent reactions, superoxide, hydrogen peroxide, and OH radicals are generated from oxygen and NADPH. The reactive oxygen species are toxic not only to the bacteria but also to surrounding tissue causing the damage observed during infections and inflammation.



**Table 1.2: Causes of neutrophilia & neutropenia**

<b>Causes of neutrophilia</b> Bacterial infection Inflammation e.g collagen disease, Crohn's disease Trauma/surgery Tissue necrosis/infarction Hemorrhage and hemolysis Metabolic, e.g diabetic ketoacidosis Primary causes: Myeloproliferative disorders. Down syndrome, hereditary neutropenia Pregnancy, stress exercise . Drugs e.g steroids, G-CSF
<b>Causes of neutropenia</b> A. Decreased Production 1. General bone marrow failure, e.g aplastic anemia, megaloblastic anemia, myelodysplasia, acute leukemia, chemotherapy, replacement by tumor 2. Specific failure of neutrophil production Congenital, e.g Kostman's syndrome Cyclical Drug induced, e.g sulphonamides, chlorpromazine, clorazil, diuretics, neomercazole, gold B. Increased destruction 1. General, e.g hypersplenism 2. specific e.g autoimmune- alone or in association with connective tissue disorder, rheumatoid arthritis "Felty's syndrome"

**Eosinophils:** Eosinophils, which make up 1-4% of the peripheral blood leukocytes, are similar to neutrophil but with some what more intensely stained reddish granules. In absolute terms, eosinophils number upto 400/ $\mu$ l. Eosinophil cells can first be recognised at the myelocyte stage. Eosinophils have a role in allergic reactions, in the response to parasites, and in the defense against certain tumors.

**Table 1.3: Causes of eosinophilia**

Allergic diseases e.g asthma, hay fever, eczema, pulmonary hypersensitivity reaction " e.g Loeffler's syndrome' Parasitic disease Skin diseases, e.g psoriasis, drug rash Drug sensitiviry Connective tissue disease Hematological malignancy e.g lymphomas, Eosinophilic leukemia Idiopathic hypereosinophilia Myeloproliferative disorders, chronic myeloid leukemia, polycythemia vera, myelofibrosis
---

**Basophils:** Basophils are seen less frequently than eosinophils; under normal conditions, less than 100/ $\mu$ l are found in the peripheral blood. Basophils have receptors for immunoglobulin E (IgE) and in the cytoplasm; characteristic dark granules overlie the nucleus. Degranulation of basophils results from the binding of IgE and allergic or anaphylactic reactions are associated with release of histamine and heparin. Basophilia can be associated with drugs, tuberculosis and ulcerative colitis. The most common setting of basophilia is in myeloproliferative disorders such as CML. Peripheral blood smear confirms basophilia and management focuses on the underlying etiology. Peripheral blood can be sent for Jak2 and bcr/abl to evaluate a myeloproliferative disorder.

**A monocyte** is influenced by hematopoietic growth factors to transform into a macrophage in the tissue. Functionally, monocytes and macrophages have phagocytosis their major role, although they also have regulatory and secretory functions.

In contrast to the granulocytic leukocytes, the promonocytic will undergo two or three mitotic divisions in approximately 2 to 2.5 days. Monocytes are released into the circulating blood within 12 to 24 hours after precursors have their last mitotic division.

Histiocytes are the terminally differentiated cells of the monocyte macrophage system and are widely distributed throughout all tissues. Langerhans' cells are macrophages present in epidermis, spleen, thymus, bone, lymph nodes and mucosal surfaces. Langerhans' cell histiocytosis is a single organ/system or multisystem disease occurring principally in childhood.

Monocytosis usually represent a malignant histiocyte disorders include monocytic variants of leukemia and some types of non-Hodgkin's lymphoma. Peripheral blood smear confirms monocytosis and management focuses on the underlying etiology. Peripheral blood can be sent for Jak2 and bcr/abl to evaluate a myeloproliferative disorder. If suspicion of a myeloproliferative disorder is high, a bone marrow biopsy is necessary.

**Lymphocytes:** Hematopoietic growth factors play an important role in differentiation into the pathway of the pre-B cell or prothymocyte. The majority of cells differentiate into T lymphocyte or B-lymphocytes. The plasma cell is the fully differentiated B cell.

The stages of lymphocyte development are the lymphoblast, the prolymphocyte, and the mature lymphocyte. Mature lymphocytes can be classified as either large or small types.

Lymphocytosis occurs in viral infection, some bacterial infections (e.g pertussis) and in lymphoid neoplasia.

Lymphopenia occurs in viral infection (e.g HIV), lymphoma, connective tissue disease, and severe bone marrow failure.

**Platelets:** Two classes of progenitors have been identified: The BFU-M and The CFU-M. The BFU-M is the most primitive progenitor cells committed to Megakaryocyte lineage.

The next stage of Megakaryocyte development is a small, mononuclear marrow cell that expresses platelet specific phenotype markers.



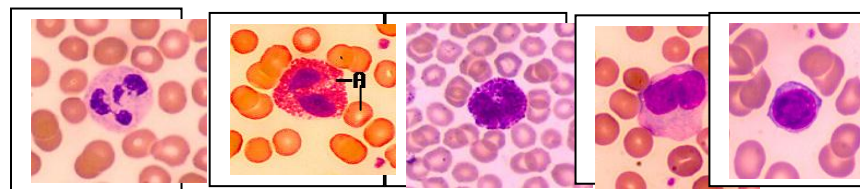
The final stage of Megakaryocyte development is recognised in bone marrow, because of their large size and lobulated nuclei. These stages are polypoid.

Megakaryocyte is largest bone marrow cells, ranging up to 160µm in size. The N: C ratio is 1:12. Nucleoli are no longer visible. A distinctive feature of the Megakaryocyte is that it is a multilobular, not multinucleated. The fully mature lobes of the Megakaryocyte shed platelets from the cytoplasm on completion of maturation. Platelet formation begins with the initial appearance of a pink colour in the basophilic cytoplasm of the Megakaryocyte and increased granularity.

Mature platelets have an average diameter of 2-4 µm, with young platelets being larger than older ones.

**Table 1.4: Characteristics of neutrophilic granulocytes**

	MYELOBLAST	PROMYELOCYTE	MYELOCYTE	METAMYELOCYTE	BAND	SEGMENTED
Size (µm)	10 – 18	14 – 20	12 – 18	10 – 18	10 -16	10 16
N:C ratio	4:1	3:1	2:1	1:1	1:1	1:1
Nucleus Shape	Oval/round	Oval/round	Oval/round	Indented	Enlarged	Distinct lobes 2-5
Nucleoli	1-5	1-5	Variable	None	None	None
Cytoplasm Inclusion:	Auer rods	None	None	None	None	None
Granules	None	Heavy	Fine	Fine	Fine	Fine
Amount	Scanty	Slightly increased	Moderate	Moderate	Abundant	Abundant
Colour	Medium blue	Moderate blue	Blue-pink	Pink	Pink	Pink



A.

B

C.

D.

E.

**Figure 1. 2: Mature leukocytes : (A) Neutrophil, (B) Eosinophil, (C) Basophil, (D) Monocyte and (E) Lymphocyte**

**Table 1.5. Characteristics of monocytes**

	Monoblast	Promonocyte	Mature monocyte
Size ( $\mu\text{m}$ )	12-20	12-20	12-18
N:C ratio	4:1	3:1-2:1	2:1-1:1
Nucleus shape	Oval/folded	Elongated/folded	Horseshoe
Nucleus	1-2 or more	0-2	None
Chromatin	Fine	Lace-like	Lace-like
Cytoplasm	Vacuoles	Vacuoles	Vacuoles
Inclusion	Variable	Variable	Common
Granules	None	None or fine	Fine dispersed
Amount	Moderate	Abundant	Abundant
Colour	Blue	Blue-grey	Blue-grey

### Hemoglobin Synthesis

It occurs in the RBC precursors from the globin polypeptide chain and heme. This synthesis stops in the mature RBCs.

Hb is a tetramer, formed of 4 polypeptide chains with a heme group attached to each chain. These polypeptides are of different chemical types. Each chain is controlled by a different gene, which is activated and inactivated in a special sequence. Alpha chain is controlled by two sets of gene (i.e. 4 genes), which are present on chromosome No. 6. Beta, Gamma and Delta chains are controlled by one set of genes (i.e 2 genes) for each chain, which are present on chromosome No. 11.

The most common Hbs are HbA ( $\alpha_2\beta_2$ , the major adult Hb), HbF ( $\alpha_2\gamma_2$ , the major fetal Hb), and HbA<sub>2</sub>, a minor adult Hb

**Table 1.6: The human hemoglobins**

Hemoglobin	Composition	Representation
A	$\alpha_2\beta_2$	95-98% of adult Hb
A <sub>2</sub>	$\alpha_2\delta_2$	1.5-3.5% of adult Hb
F	$\alpha_2\gamma_2$	Fetal Hb, 0.5-1%
Gower 1	$\zeta_2\varepsilon_2$	Embryonic hemoglobin
Gower 2	$\alpha_2\varepsilon_2$	Embryonic hemoglobin
Portland	$\zeta_2\gamma_2$	Embryonic hemoglobin

At birth, Hbf forms about 70% of the total Hb, while Hb-A forms the rest.

By 6 months of age, only trace amounts of gamma chain are synthesised and very little amounts of residual Hb-F are present. At 6-12 months age, Hb-F forms 2% of the total Hb, while Hb-A forms the rest. Hb-A<sub>2</sub> forms about 3% of the total Hb.

The release of oxygen from red cells into tissue is strictly regulated. Under normal condition, arterial blood enters tissues with an oxygen tension of 90 mmHg and hemoglobin saturation close to 97%. Venous blood returning from tissues is deoxygenated. The oxygen tension is about 40 mmHg; the oxyhemoglobin dissociation curve describes the relation between the

oxygen tensions at equilibrium. The affinity of hemoglobin for oxygen and the deoxygenation in tissues is influenced by temperature, by CO<sub>2</sub> concentration, and by the level of 2,3-diphosphoglycerate in the red cells. In the case of tissue or systemic acidosis, the oxygen dissociation curve shifted to the right and more oxygen is released. The same effect results from the uptake of carbon dioxide, which raises the oxygen tension of carbon dioxide.

The oxygen supply to peripheral tissues is influenced by three mechanisms:

1. The blood flow, which is controlled by the heart beat volume and the constriction or dilatation of peripheral vessels.
2. The oxygen transport capacity, which depends on the number of red blood cells and the hemoglobin concentration.
3. The oxygen affinity of hemoglobin

**Table 1.7: Some normal haematological values according to age**

Age	RBC/ million	Hb g/dl	Hematocrit	WBC/1000 / $\mu$ l
Cord blood	5+-1	16.5 +- 3	55+-10	18+-8
3 months	4+- 0,8	11.5 +- 2	36+-6	12+-6
6 months	4.8 +-0,7			
7 Y-12Years	4.7 +-0.7	13+- 1	38+-4	9+-4.5
Adult M	5.5+- 1	15.5 +- 2.5	47+-7	7+-3.5
Adult Female	4.8+-1	14+- 2.5	45+-5	7.5+-3.5

### Clinical Applicability of Hematopoietic Stem Cells

Stem Cell Disorders: Disorders of hematopoietic stem cells include aplastic anemia, paroxysmal nocturnal hemoglobinuria, and the various forms of acute non-lymphocytic (myelogenous) leukemia, the myeloproliferative disorders and myelodysplastic syndromes. The fact that the myeloid pluripotential stem cell is the usual locus of disease in these disorders can be inferred from the fact that the three major cell lines derived from that cell, erythrocytes, granulocytes, and platelets are often affected. Chronic myelogenous leukemia is believed to arise at a still more primitive level, that of the multipotential stem cell; lymphoid as well as myeloid cells appear to be involved in the neoplastic process, and some patients with this disease undergo transformation to acute lymphocytic leukemia. Direct evidence that these disorders originate at the level of the pluripotential stem cell has been obtained by chromosome analysis and by studies of black female patients who are heterozygotes for two isotypes of the enzyme glucose 6-phosphate dehydrogenase.



## Growth Factors as Therapeutic Agents

Now that purified growth factors can be produced in large quantities, clinical trials are taking place to examine their ability to stimulate hematopoiesis in patients. The recombinant GM-CSF can offset the neutropenia that follows intensive chemotherapy for malignant disorders. This agent also hastens recovery of the peripheral blood counts after bone marrow transplantation. Recombinant reverses the anemia of chronic renal insufficiency.

The effective use of **granulocyte-colony-stimulating factor** (G-CSF) as a part of a therapeutic maneuver enabling otherwise lethal therapy to be given for an uncontrollable disorder. G-CSF and GM-CSF (**granulocyte macrophage colony** stimulating factor), originally derived from human leukocytes or fibroblasts, are now manufactured by recombinant techniques. They have been shown to be involved in the process of proliferation and differentiation of bone marrow progenitors. GM-CSF is a growth factor for both macrophages and neutrophils and is presumably active slightly earlier in the identification pathway than G-CSF, which is restricted to the granulocyte cell line. Both can be given intravenously or subcutaneously depending upon circumstances. Bone pain is the major complaint registered by patients who receive G-CSF or GM-CSF, but fever, malaise, and discomfort at the injection site also occur. At higher doses (e.g.  $>32\mu\text{g/kg}$ ) the side effects are worsened, and a capillary leak syndrome is well known to occur with GM-CSF.

Erythropoietin (Epo) is currently available for therapeutic use, and in anephric individuals it has been demonstrated to raise levels of hemoglobin from a base of about 60 gm/L to 100 gm/L. It has value in secondary chronic anemia, and also been tried with variable results in some cases of myelodysplasia. However, it is not clear exactly how Epo exerts its effect on red cell production, but it likely acts on the immediate post-stem cell daughter cells, shifting them to the red cell line and also shortening the RBC period of maturation within marrow itself. Epo receptors are also present on the surface of marrow erythroid precursor's megakaryocytes and fetal liver cells.

### Identification of Cells

In identifying a cell, the technologist should think in the following terms:

1. What is the size of the cells?
  - a. Small
  - b. Medium
  - c. Large
2. What are the characteristics of the nucleus?
3. What are the characteristics of the cytoplasm?

When attempting to identify cells, it is important to note the degree to which the cells take up the stain.

**Shape:** A normal RBC is a biconcave disc about 2 microns in thickness.

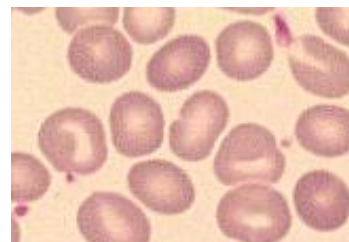
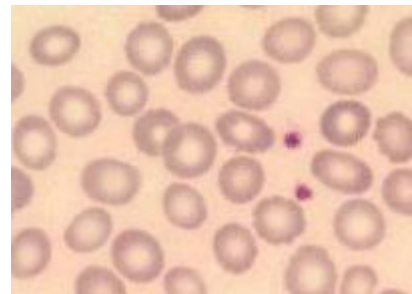
**Size:** The mean corpuscular diameter is 6.7 - 7.9 microns (average 7.2).

The variation from normal can be classified as:

1. Variation in size
2. Variation in shape
3. Alteration in color
4. Inclusions in the erythrocytes
5. Alteration in RBC distribution.

### VARIATION IN SIZE "ANISOCYTOSIS"

1. **Microcytes:** are small cells less than 6.7 micron
  - Iron deficiency anemia
  - Thalassemia
  - Sideroblastic anemia
  - Lead poisoning
  - Chronic disease
2. **Macrocytes** are abnormally large cell (8-12 micron)
  - Deficiency of Vitamins B12 or Folate.
  - Hypothyroidism
  - Liver disease
  - Alcohol
  - Smoking
3. **Megaloblasts:**  
are extremely large (12 to 25 micron)
  - Vitamin B12 deficiency
  - Folate deficiency
  - Drugs:  
Methotrexate,  
Cyclophosphamide,  
Nitrous oxide, Arsenic



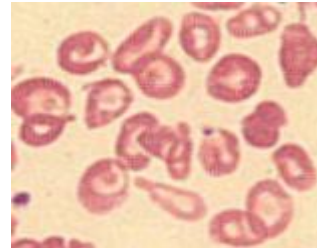


## VARIATION IN SHAPE "POIKILOCYTOSIS"

### 1. **Poikilocytes** are pear-shaped

RBC denoting destruction.

Poikilocytosis caused by a defect in the formation of red cells. It may be seen in pernicious anemia, IDA, congenital hemolytic anemia and many other types of anemia



### 2. **Acanthocytes** has multiple thorny spike-like projections that are irregularly distributed around the cellular membrane and vary in size.

found in:

- Liver cirrhosis
- Hepatic hemangioma
- Neonatal hepatitis
- Postsplenectomy
- Retinitis pigmentosa



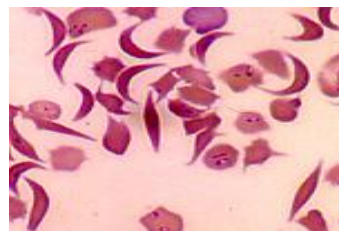
### 3. **Spherocyte** is red cells of smaller diameter but more thicker than normal cells. They represent RBC imbedding waters and so become more fragile than normal.

- ABO hemolytic disease of newborn.
- Acquired hemolytic anemia
- Congenital spherocytosis
- Blood transfusion reaction
- DIC



### 4. **Sickle cells** are red cells due to the presence of HbS.

They can only be seen in wet film (Sickling test) and appear as sickle shaped cells when the oxygen tension is reduced in the preparation or on addition of reducing substances as sodium metabisulfate.

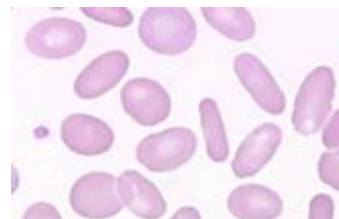


### 5. **Ovalocytes** : are elliptical cells,

oval biconvex cells. It is an inherited anomaly. It may

be found in:

- H. elliptocytosis
- Thalassemia
- S.C.A



6. **Elliptocytes** are generally narrower and more elongated than megalocyte. These cells have a rod, cigar or sausage shape. Elliptocytosis associated with:

- Anemia of malignancy
- Hemoglobin C disease
- H. Elliptocytosis
- IDA, Pernicious anemia
- Sickle Cell Trait
- Thalassemia

7. **Crenated erythrocyte (Echinocytes):**

Crenated erythrocytes have puckered outer edges. Crenated RBC may be produced in

- Blood smear which dries slowly
- They are often found in
- various portions of the blood smear.

8. **Burr cells** are erythrocytes having one or more spine projections of cellular membrane.

Increased in:

- Anemia
- Bleeding gastric ulcers
- Gastric carcinoma, Peptic ulcer
- Renal insufficiency, uremia
- Pyruvate kinase insufficiency

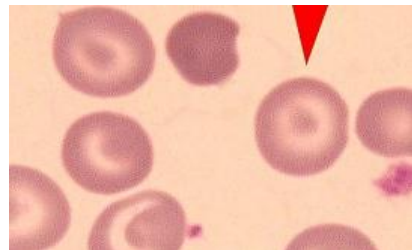
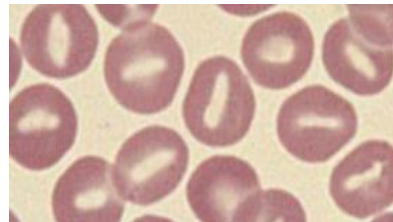
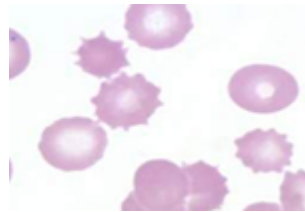
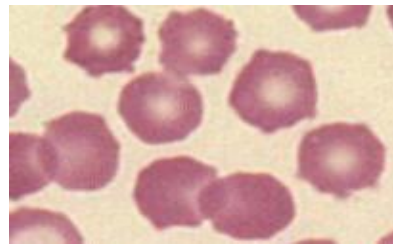
9. **Stomatocytes** have a slitlike opening that resembles a mouth. Increase stomatocytes in acute alcoholism, alcoholic cirrhosis,

H.septherocytosis infection mononucleosis, lead poisoning and malignancies.

10. **Target cell (codocytes)** are erythrocytes that resemble a shooting target. A central red bull's eye is surrounded by a clear ring and then an outer red ring.

Clinically, Target cells are seen in:

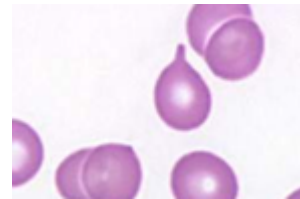
- Hemoglobinopathies
- Hemolytic anemia, Hepatic disease and IDA & Postsplenectomy



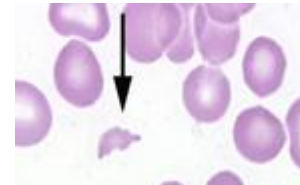


11. **Teardrop cell** (dacrocytes) is usually

smaller than erythrocytes. Teardrop cell resemble tears. This cellular abnormality is associated with homozygous beta thalassemia, myeloproliferative syndrome, pernicious anemia



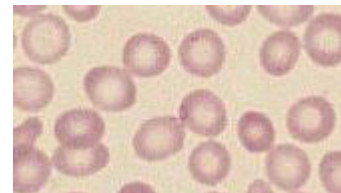
12. **Schistocytes** are red blood cell fragments and may occur in microangiopathic hemolytic anemia, uremia, severe burns, and hemolytic anemia caused by physical agents, as in DIC



#### ALTERATION IN ERYTHROCYTE COLOUR: ANISOCROMIA

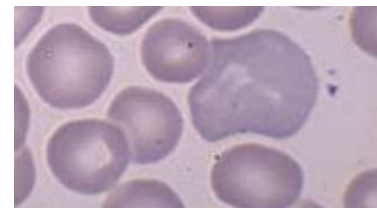
**Hypochromia** term used when central pallor exceeds one third of the cells diameter or the cell has a pale overall appearance.

It is clinically associated with iron deficiency anemia.



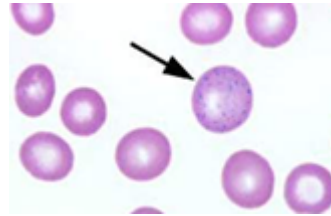
**Polychromatophilia** is used if a nonnucleated erythrocyte has a faintly blue orange colour when stained with Wright stain. This cell lacks the full amount of hemoglobin and the blue colour is due to diffusely distributed residual RNA in the cytoplasm.

The polychromatophilic erythrocyte is larger than a mature erythrocyte. If stained with a supravital stain, a polychromatophilic erythrocyte would appear to have a threadlike netting within it and would be called a reticulocyte.

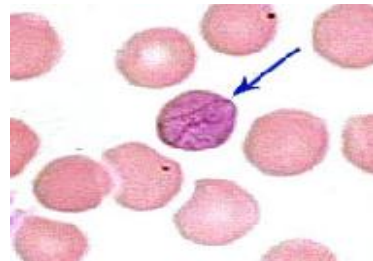


## VARIATION IN ERYTHROCYTE INCLUSIONS

1. **Basophilic stippling** appears as deep blue granulations of variable size and number. Stippling represents granules composed of pathological aggregation of ribosome and RNA that are precipitating during the process of staining of a blood smear. Stippling is associated clinically with lead poisoning and severe anemia and thalassemia.



2. **Cabot rings** are ring-shaped, figure-eight or loops shaped structures. These inclusions represents nuclear cabot rings can be seen in lead poisoning and pernicious anemia

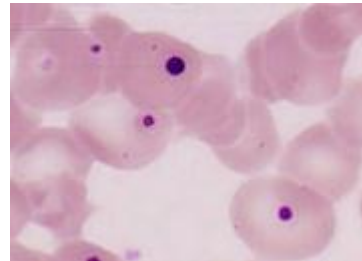


3. **Howell-Jolly bodies** are round solid staining, dark-blue to purple inclusions. If presents, cells contain only one or two H-J bodies. Most frequently seen in mature RBC.

They are DNA remnant.

H-J bodies is associated with:

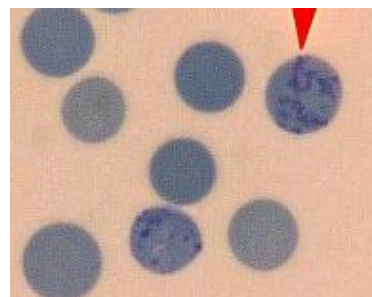
- Hemolytic anemia
- Pernicious anemia
- Postsplenectomy
- Physiological atrophy of spleen



4. **Papenheimer bodies** (siderotic granules) are dark-staining particles of iron in the erythrocyte that are visible with a special iron stain- Prussian blue. They appear as blue dots and represents ferric ions.

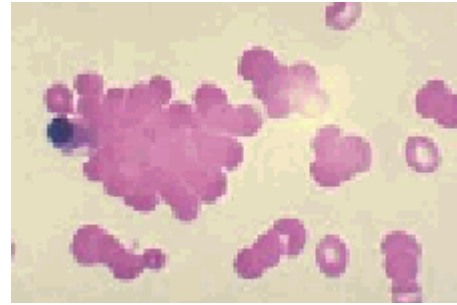
Clinically they are associated with:

- Iron loading anemia
- Hyposplenism
- Hemolytic anemia



## ALTERATION IN ERYTHROCYTE DISTRIBUTION

1. **Agglutination** is due to presence of antibodies reacting with antigens on the erythrocyte. e.g. MAHA, AIHA



2. **Rouleaux formation** is associated with the presence of cryoglobulin. E.g. Multiple myeloma and Waldenström Macroglobulinemia



Table 1.8 Red blood cell morphology grading chart

Morphology	Grade As
Polychromatophilia Tear drop RBC Acanthocytes Schistocytes Spherocytes	1+ = 1 to 5/field 2+ = 6 to 10/field 3+ = > 10/field
Poikilocytosis Ovalocytes Elliptocytes Burr cells Bizarr-shaped RBC Target cells Stomatocytes	1+ = 3 to 10/field 2+ = 11 to 20/field 3+ = > 20/field
Rouleaux	1+ = aggregate of 3 to 4 RBCs 2+ = aggregate of 5 to 10 RBCs 3+ = Numerous aggregates with only a few free RBCs
Sickle Cells Basophilic stippling Pappenheimer bodies Howell-Jolly bodies	Grade as positive only



## REVIEW QUESTIONS

1. The primary site of hematopoiesis in fetus between the 10<sup>th</sup> and the 30<sup>th</sup> week of gestation is the:
  - a. Spleen
  - b. Bone marrow
  - c. Thymus
  - d. Liver
2. Active blood cell producing marrow begins to regress in the fourth year of life and replaced by:
  - a. Bone
  - b. Fat
  - c. Fibrous tissue
  - d. Collagen
3. The red cell progenitor that requires relatively large amount of erythropoietin to respond is the
  - a. CFU-GEMM
  - b. BFU-E
  - c. CFU-E
  - d. Pronormoblast
4. The two cell types that produce their own growth factors are:
  - a. Neutrophils and monocytes
  - b. Neutrophils and lymphocytes
  - c. Neutrophils and eosinophils
  - d. Lymphocytes and monocytes
5. The following cells are of the myeloid cell line except
  - a. Platelets
  - b. T-lymphocyte
  - c. Promyelocyte
  - d. Monocyte
6. The major erythrocyte production site is the
  - a. Bone marrow
  - b. Kidney
  - c. Liver
  - d. Spleen
7. The normal red cell
  - a. Is biconvex
  - b. Has only hemoglobin within the cell membrane
  - c. Has a normal MCV of 80-100 fl
  - d. Is an erythroblast?
8. A normal mature erythrocyte has a life span of
  - a. 8.2 hours
  - b. 5 days
  - c. 28 days
  - d. 120 days
9. Howell-Jolly bodies are clinically seen in the following except:
  - a. Hemolytic anemia
  - b. Iron deficiency anemia
  - c. Pernicious anemia
  - d. Postsplenectomy

10. The major site for removal of normal, aged erythrocytes is the
  - a. Bone marrow
  - b. Liver
  - c. Kidney
  - d. Spleen
11. The elliptocyte is prominent morphology in :
  - a. Myeloid metaplasia
  - b. Hemolytic anemia
  - c. Iron deficiency anemia
  - d. Sickle cell anemia
12. The blood smear of a patient with a prosthetic heart valve may show:
  - a. Target cells
  - b. Burr cells
  - c. Schistocyte
  - d. Elliptocyte
13. How would a cell that has a diameter of 9  $\mu\text{m}$  and an MCV of 104 be classified?
  - a. Macrocyte
  - b. Microcyte
  - c. Normal
  - d. Either normal or slightly microcytic
14. Which type of red cell inclusion is a DNA remnant?
  - a. Heinz bodies
  - b. Howell-Jolly bodies
  - c. Pappenheimer bodies
  - d. Cabot rings
15. In a patient with an MCHC > 36%, one would expect to observe:
  - a. Target cells
  - b. Spherocytes
  - c. Elliptocytes
  - d. All of them
16. A microcytic cell can be described as possessing:
  - a. A thin rim of hemoglobin
  - b. A blue-gray color
  - c. A size of less than 7  $\mu\text{m}$
  - d. An oval shape
17. Basophilic stippling is composed of
  - a. DNA
  - b. Precipitated stain
  - c. Denatured hemoglobin
  - d. RNA
18. Which inclusion cannot be visualized on Wright's stain?
  - a. Basophilic stippling
  - b. Pappenheimer bodies
  - c. Howell-Jolly bodies
  - d. Heinz bodies

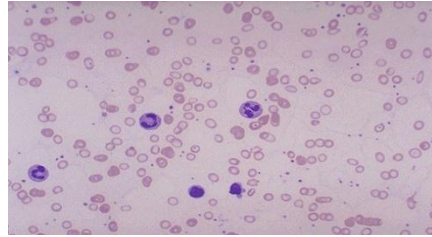
19. Which morphologies would be prominent on a smear of a patient with severe liver disease?
- Target cells, macrocytes
  - Microcytes, elliptocytes
  - Schistocytes, bite cells
  - Sickle cell, crystals
20. The progression of erythropoiesis from prenatal life to adulthood is:
- Yolk sac – red bone marrow – liver and spleen
  - Yolk sac – liver and spleen – red bone marrow
  - Red sac – liver and spleen – yolk sac
  - Liver and spleen – yolk sac – red bone marrow
21. Normal adult hemoglobin has
- Two alpha and two delta chains
  - Three alpha and one beta chains
  - Two alpha and two beta chain
  - Two beta and two epsilon chain
22. The number of heme groups in a hemoglobin molecule is:
- One
  - Two
  - Three
  - Four
23. Which of the following is the term for erythrocytes resembling “a stack of coins” on thin sections of a peripheral blood smear?
- Anisocytosis
  - Poikilocytosis
  - Agglutination
  - Rouleaux formation

Questions 24 through 30, match the following

- |                          |                               |
|--------------------------|-------------------------------|
| 24. Basophilic stippling | a. Pernicious anemia          |
| 25. H-Jolly bodies       | b. G6PD deficiency            |
| 26. Heinz bodies         | c. Lead poisoning             |
| 27. Pappenheimer bodies  | d. Iron deficiency anemia     |
| 28. Acanthocytes         | e. Iron loading anemia        |
| 29. Spherocytes          | f. Abetalipoproteinemia       |
| 30. Microcytes           | g. Blood transfusion reaction |



## ANEMIA



2

Anemia a decrease in red cell mass is also defined as a decrease in the hemoglobin concentration or decrease in the hematocrit when compared with a normal group.

Anemia is functionally defined as a decrease in the competence of blood to carry oxygen to tissues thereby causing tissue hypoxia.

The symptoms of anemia depend upon the degree of reduction in the oxygen-carrying capacity of the blood, the change in the total blood volume, the rate at which these changes occurs, the degree of severity of the underlying disease contributing to the anemia, and the power of the cardiovascular and hematopoietic systems to recuperate and compensate.

To understand how anemia develops, it is necessary to understand normal erythrocyte kinetics. Total erythrocyte mass in the steady state is equal to the number of new RBCs produced per day times the erythrocyte life span (100-120 days):

$$\text{Mass (M)} = \text{Production (p)} \times \text{Survival (s)}$$

Thus, the average 70-Kg man with 2 litres of erythrocytes must produce 20 ml of new erythrocytes each day to replace the 20 ml per day normally lost due to cell senescence.

$$\frac{2000 \text{ ml (M)}}{100 \text{ days (S)}} = 20 \text{ ml / day (p)}$$

Using this formula it can be seen that if the survival time of the erythrocyte is decreased by half, the bone marrow must double production to maintain a constant mass.

$$\frac{2000 \text{ ml (M)}}{50 \text{ days (S)}} = 40 \text{ ml / day (P)}$$

New red cells are released from marrow as reticulocytes; thus a doubling of the absolute count reflects the increased production. The marrow can compensate for decreased survival in this manner until production is increased to a level 5 to 10 times normal, which is the maximal functional capacity of the marrow.

### Diagnosis of Anemia

- History
- Physical Examination
- Clinical Laboratory

### Laboratory Investigations

1. RBC count, Hematocrit and Hemoglobin
2. Red Cell Indices.
3. Reticulocytes count
4. Blood smear examination
5. Bone marrow examination

MCV: Indices the average volume of individual red cells in the femtoliters (fl) by the use automated cell counter is manually calculated:  $MCV (FL) = \frac{\text{Hematocrit (L/L)}}{\text{RBC count (X10}^{12}/L)}$

RBC count (X10<sup>12</sup>/L)

Example: A patient has a hematocrit of 0.45L/L and an RBC of 5X10<sup>12</sup>/L

$$MCV = \frac{0.45}{5 \times 10^{12}} = 90 \times 10^{-15} \text{ or } 90 \text{ fl}$$

The MCV is used to classify cells as normocytic (80-100), microcytic cells have (< 80) and macrocytic (>100 fl).

Mean Corpuscular hemoglobin Concentration (MCHC): MCHC is the average concentration of hemoglobin in grams in a decilitre of red cells.

The MCHC is calculated from the hemoglobin and hematocrit as follows:

$$MCHC \text{ g/dl} = \frac{\text{Hemoglobin (g/dl)}}{\text{Hematocrit (L/L)}}$$

Example: A patient has a hemoglobin amount of 15 g/dl and a hematocrit of 0.45 (L/L)

$$MCHC = \frac{15 \text{ g/dl}}{0.45 \text{ L/L}} = 33 \text{ g/dl}$$

Normal MCHC is 31 to 36% normochromic

< 31% Hypochromic

> 36% Hyperchromic

Mean Corpuscular Hemoglobin (MCH) is a measurement of the average weight of hemoglobin in individual red cells. The MCH may be calculated as follows:

$$MCH (pg) = \frac{\text{Hemoglobin (g/d)}}{\text{RBC count (X10}^{12}/l)} \times 10$$

The normal MCH should always correlate with the MCV and MCHC

< 27 pg microcytic    > 34 pg macrocytic

### The Hematological Rules of Three and Nine

#### The First Rule of Three

Red cell count (in million) X 10<sup>12</sup> /L X3 = Hemoglobin/dl

This rule expresses the normal ratio of hemoglobin to red cells. It applies to all hemoglobinized cells regardless of whether or not the patient is anemic. Such normalized cells are found in anemias of various causes, so if this rule is found to fit an anemia, and leukemia. If the test results do not fit this rule, the anemia, if present, may be broadly subclassified into that possessing increased numbers of red cells in proportion to the total hemoglobin (e.g.



hypochromic microcytic anemias) and that with a disproportionately low red cell count in relation to the hemoglobin (e.g. macrocytic anemia). Hypochromasia, commonly seen in severe iron deficiency anemia, can usually be detected on the peripheral blood smear when the hemoglobin is less than 10 g/dl.

### **The Second Rule of Three**

This rule is an expression of normal red cell relationship, and abnormalities of rule 2 are indicative of pathological states. For example, moderate to severe iron deficiency anemia usually is indicated when the hemoglobin is disproportionately lower than the hematocrit, producing an abnormal result when rule 2 is applied. The hypochromia of thalassemia frequently does not violate this rule, as there is often agreement between the hemoglobin and the hematocrit. Consequently, rule 2 holds in such situations. Thus this rule is most often used for corroborative rather than diagnostic purposes. Its main use is as a check on the validity of the test results as part of a quality control program.

### **The Rule of Nine**

If the normal ratio of the red cell count to the hemoglobin and that of hemoglobin to hematocrit is known, a rule of nine can be calculated that express the numerical relationship of the hematocrit to the red cell count.

$$\text{Red cell count (} 10^{12} / \text{L} \times 9 = \text{Hematocrit (\%)} )$$

This is derived from the two rules of three as follows:

1. Red cell count  $\times 3 =$  Hemoglobin
2. Hemoglobin  $\times 3 =$  Hematocrit, that is Hemoglobin = Hematocrit/3
3. Red cell count  $\times 3 =$  Hemoglobin, that is, red cell count  $\times 9 =$  Hematocrit

### **The Peripheral Blood Smear**

The peripheral blood smear is the most practical diagnostic tool for the hematologist. A drop of blood is applied against slide that is subsequently stained with polychrome stains

(Wright-Giemsa) to permits identification of the various cell types. These stains are mixtures of basic dyes (methylene blue) that are blue and acidic dyes (eosin) that are red. As such, acid components of the cell (nucleus, cytoplasmic RNA, basophilic granules) stain blue or purple, and basic components of the cell (hemoglobin, eosinophilic granules) stain red or orange. In addition to the polychrome stains, monochrome stains are sometimes used to visualize young red cells (reticulocyte stain), denatured hemoglobin (Heinz body stain) or cellular iron (Prussian blue stain).

The blood smear is used to assess red cell size/shape; white cell appearance and differential; abnormal cells; platelet size and morphology; detection of parasites, e.g. malaria. The smear may suggest a diagnosis, e.g. type of anemia, presence of malaria, leukemia and myelodysplasia.

### **Bone Marrow Examination**

Marrow examination complements clinical and laboratory in determining the cause of anemia, leukopenia, leukocytosis, thrombocytopenia and thrombocytosis, as well as contributing of lymphoproliferative disease and various solid tumors.

Smear on approximately 12 slides should be prepared to enable 5 slides to be stained with Wright-Giemsa stain for Hemosiderin and ringed sideroblast evaluation. Additional aliquots of 3-5 ml. of anticoagulated marrow for each ancillary study are prepared.

**Table 2.1: Bone marrow specimen collection**

Study	Anticoagulant
Molecular Diagnostic studies	E.D.T.A
Flow Cytometry	Heparin
Cytogenetics	Heparin

### **Biopsy**

Following fixation, decalcification and paraffin embedding, staining is conventionally accomplished with Hematoxylin and Eosin stain (H&E).

### **Reporting**

- A. The report should include a review of the peripheral blood smear including red cell, leukocyte and platelet morphology, as well as adequacy, etc.
- B. The report should include an assessment of the marrow aspirate including adequacy and technical quality of the specimen, conclusion and diagnosis.

### **Review of the marrow including**

1. Marrow differential count of 500 Nucleated cells
2. Comments regarding cellularity
3. M:E ratio
4. Type of Erythropoiesis (Normoblast, Megaloblastic, Dysplastic)
5. Hemosiderin content – Grade 0-6
6. Evaluation of Ringed sideroblasts if appropriate
7. Myeloid series comments
8. Lymphoid elements comments
9. Plasma cell elements
10. Megakaryocytes Series- (Normal, Increased, Decreased, Dysplastic)

### **Bone Marrow analysis**

1. The interpretation correlates observations made from the marrow examination with data obtained from the complete blood count. It is a good policy to insist on a concurrent complete blood count with each bone marrow aspirate.
2. More than one diagnosis may be possible for each case.
3. Each bone marrow aspirate may have a cytologic and / or etiologic diagnosis.
4. The cytologic interpretation utilizes the M/E ratio and the differential cell count to provide diagnostic data for the clinician.

**Table 2.2 Cytology interpretation of bone marrow aspiration**

M:E ratio	Complete Blood Count	Cytologic Diagnosis
Increased	Anemia Normal Leukogram	Erythroid Hypoplasia
Increased	Anemia Leukocytosis	Erythroid Hypoplasia or Myeloid Hyperplasia or both
Decreased	Leukopenia Normal Erythrogram	Myeloid Hypoplasia
Decreased	Leukopenia Polycythemia	Erythroid Hyperplasia or Myeloid Hypoplasia or Both

5. The interpretation of bone marrow aspirate results may be heavily influenced by the patient's history and alterations in sequential hemograms.

### **Classification of Anemia**

#### **Morphologic classification**

This morphologic classification is helpful since the MCV and MCHC are known at the time anemia is diagnosed, and certain causes of anemia characteristically produce a specific size of red cells (large, small or normal) and a specific type of hemoglobin content (normal or abnormal). The general categories of a morphologic classification include macrocytic-normochromic, normocytic-normochromic, and microcytic hypochromic.

#### **Functional classification**

Considering that the normal bone marrow compensatory response to decreased peripheral blood hemoglobin levels is an increase in erythrocyte production, persistent anemia may be expected as the result of three pathophysiologic mechanisms:

a. Proliferation defect   b. Maturation defect   c. Survival defect

#### **Classification of Anemia according to Severity:**

1. Mild anemia   Hb < 11 - 8 g/dl
2. Moderate   Hb < 8 - > 5g/dl
3. Severe anemia   Hb < 5 g/dl

#### **Classification of RBC Distribution Width (RDW)**

RDW is the mathematical expression of variation in size of RBC (or anisocytosis) which is calculated by the cell counters. The RDW is the coefficient of variation of the normally gaussian curves shaped RBC volume distribution histogram. The RDW is determined by dividing the standard deviation of the mean corpuscular volume (MCV) by MCV and multiplying by 100 to convert to a percentage value. Thus the RDW is a quantitative measure of the size variation of circulating RBCs. The normal value for RDW is 12-15 %.



**Table 2.3: Classification of RBC distribution width (RDW)**

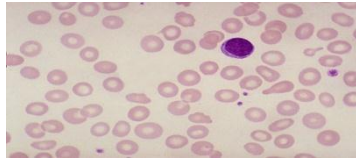
	MCV low	MCV normal	MCV high
RDW normal	Microcytic-Homogenous	Normocytic-Homogenous	Macrocytic-Homogenous
	Heterogenous Thalassemia Chronic disease	Normal Chronic disease Chronic liver disease Transfusion Chemotherapy CML Hemorrhage H. Spherocytosis	Aplastic anemia Preleukemia
RDW - high	Microcytic Heterogenous	Normocytic - Heterogenous	Macrocytic - Heterogenous
	IDA S.β-Thalassemia Hemoglobin H R.cell fragmentation	Early iron or folate deficiency Sickle cell disease Myelofibrosis Sideroblastic anemia	Folate deficiency Vitamin B12 def. IHA Cold agglutination

**Table 2. 4: Classification of anemia using morphology, RPI and iron status .**

Type of anemia	RPI	Causes
Macrocytic	>2  <2  <2	Survival defect <ul style="list-style-type: none"> <li>• Hemolysis, Hemorrhage</li> </ul> Nuclear maturation defect (megaloblastic) <ul style="list-style-type: none"> <li>• B12 and folate deficiency.</li> <li>• Drug induced</li> <li>• Congenital</li> <li>• myelodysplasia</li> </ul> Non-megaloblastic <ul style="list-style-type: none"> <li>• Liver disease, Alcoholism</li> <li>• Endocrinopathy and aplasia</li> </ul>
Normocytic Normochromic	>2  <2	Survival defect <ul style="list-style-type: none"> <li>• Hemolysis and Hemorrhage</li> </ul> Proliferation defect Marrow damage or replacement Stem cell defects Renal disease and endocrinopathies Chronic disease and liver disease
Microcytic Hypochromic	Iron in serum	Decreased: Cytoplasmic maturation defect <ul style="list-style-type: none"> <li>• Iron deficiency anemia/ Ch. disease</li> </ul> Normal or increased Cytoplasmic maturation defect: <ul style="list-style-type: none"> <li>• Hemoglobinopathies</li> <li>• Sideroblastic anemia</li> <li>• Lead intoxication</li> <li>• Porphyria</li> </ul>

## IRON DEFICIENCY ANEMIA

# 3



Iron deficiency anemia is the most common nutritional deficiency in the world. In order to understand the symptoms, etiology and treatment of this anemia, it is necessary to review normal iron and heme metabolism.

### Iron Metabolism

The total body iron content of the normal adult varies from 3 to 5 g depending on the sex and weight of the individual. It is great in males than in females, and it increases roughly in proportion to body weight. Hemoglobin iron constitutes approximately 60-70% of the total body iron. The absolute amount is varying from 1.5 to 3 grams. At the end of their life span, the aged red cells are phagocytosed by cells of the RES. Nearly all the iron derived from the breakdown of hemoglobin is released into the circulation bound to the iron binding protein, transferrin, and is re-utilised by marrow erythroblasts for hemoglobin synthesis.

### Storage

The amount of storage iron has been estimated to be about 1000-2000 mg in the healthy adult male, and less in the female. Storage irons occur in two forms- ferritin and hemosiderin. Ferritin is normally predominant. In the normal person, storage iron is divided about equally between the reticuloendothelial cells (mainly in spleen, liver and bone marrow), hepatic parenchyma cells and skeletal muscle. Hemosiderin, the main storage form in reticuloendothelial cells, is more stable and less readily mobilized for hemoglobin formation than ferritin, which predominates in hepatocytes. In states of iron overload, hemosiderin increases to a great degree than ferritin and became the dominant storage form.

### Plasma (transport) Iron

Between 3 and 4 mg iron are present in the plasma, where it is bound to a specific protein, transferrin, a B-globulin of molecular weight 88000 that is synthesized in the liver. Each molecule of transferrin binds one or two atoms of ferric iron.

The function of transferrin is the transport of iron. It is the means by which iron absorbed from the alimentary tract is transported to the tissue stores, from tissue stores to bone marrow erythroblasts, and from one storage site to another. When transferrin reaches the storage sites or the bone marrow, it attaches to specific receptors on the cells and liberates its ferric ions, which pass into the cell to be stored or utilized. The total amount of transferrin in the plasma is about 8 g in the extracellular fluid. The level of serum iron in normal subjects averages about 20  $\mu$ mol (men > women).

Transferrin is a glycoprotein present in the serum in concentration, which enables it to combine with 44-80  $\mu\text{mol}$  of iron per litre. This value is known as the total iron binding capacity of the serum.

Serum ferritin concentration in adult's range between 15-300  $\mu\text{g/l}$  and the mean level in men and women are 123  $\mu\text{g/l}$  and 56  $\mu\text{g/l}$ . In iron deficiency, concentration is less than 12  $\mu\text{g/l}$ . In iron overload, levels are very high.

Serum ferritin increased in: infections, inflammations, malignancy (lymphomas, leukemias) and liver diseases.

### **Absorption**

The small intestine is highly sensitive to repletion of iron stores and rapidly corrects imbalance by decreasing or increasing absorption. The daily intake of a normal adult on a mixed western type diet contains 10-20 mg iron of which 10% or somewhat less is absorbed. Absorption is greater requirement due to menstrual loss and childbearing.

The chief dietary sources are meats (liver, kidney) egg yolk, green vegetables and fruit milk.

Heme iron present in the hemoglobin and myoglobin of meat is well absorbed.

Non-heme iron is released from food as ferric or ferrous ions by the action of acid in the stomach. It is absorbed only in the ionic state and almost exclusively as the ferrous form. Ferric ions are soluble at low PH. Ferrous ions are more soluble under these conditions.

Absorption of iron is most efficient in the duodenum and proximal jejunum, following entry into the cell, depending on the body requirement for iron; a proportion is rapidly transferred across the cell and on to the portal circulation for distribution to tissue iron stores. Most of the remaining iron in the mucosal cell combines with apoferritin to form ferritin. The ferritin containing cells are exfoliated from the mucosal surface at the end of their 2-3 lifespan, and the iron is lost in the faeces.

### **Excretion**

The amount of iron lost from the body per day is between 0.5 and 1.0 mg under physiological conditions. The rate of loss is relatively constant and independent of intake.

### **Iron Imbalance**

Under normal circumstances, iron absorption exceeds iron excretion. The diet normally contains 10-20 mg of iron, of which 10% is absorbed.

Uptake varies from 1-2 mg per day. Basal loss ranged from 0.5 to 1 mg per day. Menstrual iron loss is monthly 15-25 mg that mean between 0.5 and 1 mg per day. (28 days). The daily absorption necessary to compensate for daily loss is 0.5 to 1mg in males and about twice this amount i.e. 1-2 mg in female during the reproductive period of life.

The daily iron requirement for hemoglobin synthesis is 20-25 mg. It has been pointed out that the body conserves its iron stores by reutilising the iron derived from the breakdown of the hemoglobin from aged red cells. In normal individual red cell destruction and formation take place at almost identical rates. Normal males are a state of positive iron balance. Female of childbearing age, the positive balance is only very slender, because of the



additional loss by menstruation. Thus, a moderate increase in menstrual loss, especially if associated with impaired intake, can easily induce negative iron balance.

### Causes

1. Increased physiological requirements;
  - a. Growth: IDA is more common in children between age of 6 month and 2 years, and from 11 to 16 years due to spurts of growth during these periods.
  - b. Menstruation: Anemia common in adult menstruating females.
  - c. Pregnancy: During pregnancy anemia is almost universal.
2. Pathological blood loss:
  - a. Menorrhagia- Antepartum and postpartum bleeding.
  - b. Gastrointestinal tract:
    - Bleeding piles
    - Drugs: Aspirin, Indomethacin, Butazolidin and Corticosteroids
    - Peptic ulcer
    - Intestinal infestations and infections: Ankylostoma, whipworm, chronic colitis
    - due to amoebic or bacillary infections and giardiasis,
    - Miscellaneous: Cirrhosis of liver, hiatus hernia, diverticulosis of colon, TB bowels,
    - Crohn's disease, ulcerative colitis and malignancy of bowels.
  - c. Malabsorption: Coeliac disease, postgastrectomy and atrophic gastritis.
  - d. Urinary Tract: Recurrent hematuria and hemoglobinuria
  - d. Others
    - Regular blood donation
    - Recurrent epistaxis
    - Recurrent hemoptysis.
    - Pulmonary hemosiderosis
    - Hereditary telangiectasis
3. Nutritional defect
  - Low iron intake
  - Inhibitors in diet
4. Excess iron loss
  - Exfoliative dermatitis, PNH, gastritis, GI infection and intravascular hemolysis.

### Pathophysiology

Iron deficiency develops in sequential stages over a period of negative balance (loss exceeds absorption).

Since Fe is absorbed with difficulty, most people barely meet their daily requirements added losses due to menstruation (mean 0.5 mg/day), pregnancy (0.5 to 0.8 mg/day), lactation (0.4 mg/day) and blood loss due to disease or accident readily lead to Fe deficiency. Fe depletion results in sequential changes or stages.

**Stage I (Depletion of iron stores):** Fe loss exceeds intake: with this negative Fe balance, storage Fe (represented by bone marrow Fe content) is progressively depleted. Although the Hb and serum Fe remain normal, the serum ferritin concentration falls to <20 ng/ml. As storage Fe decreases,

there is a compensatory increase in absorption of dietary Fe and in the concentration of transferrin (represented by a rise in Fe-binding capacity).

**Stage II (Impaired erythropoiesis):** Exhausted Fe stores cannot meet the needs of the erythroid marrow, while the plasma-transferrin level increases, the serum-Fe concentration declines, leading to a progressive decrease in Fe available for RBC formation. When serum falls to  $< 50 \mu\text{g/dl}$ , and transferrin saturation to  $< 16\%$ , erythropoiesis is impaired. The serum ferritin receptor concentration rises ( $> 8.5\text{mg/l}$ )

**Stage III (Anemic stage):** The anemia with normal appearing RBCs and indices is defined.

**Stage IV:** Microcytosis and then hypochromia are present. The most significant finding is the classic microcytic hypochromic anemia.

**Stage V:** Fe deficiency affects tissue, resulting in symptoms and signs.

## Clinical and laboratory features

The onset is insidious. In early stage, there is no clinical manifestation. But with complete depletion of iron stores, anemia develops and clinical symptoms appear. Symptoms such as weakness and lethargy are considered to be related to hypoxia cause by the decrease in hemoglobin.

A variety of other abnormalities may occur from an absence of tissue iron in iron-containing enzymes. These include koilonychia (concavity of nail) glossitis, pharyngeal webs, muscle dysfunction, impaired thermogenesis and gastritis.

The most common dysphagia described in patients with IDA includes ice-eating (phagophagia), dirt eating (geophagia), and starch eating (amylophagia).

**Peripheral blood Picture:** The blood picture is well-developed iron deficiency is microcytic (MCV 55 to 74 fl), hypochromic (MCHC 22 to 31 g/dl, MCH 14 to 26 pg). When the anemia is mild, the morphologic aspects of the red blood cells are little affected. Microcytosis and anisocytosis are usually the first morphologic signs to develop even before anemia develops.

The blood film demonstrates progressive poikilocytosis. The most frequent poikilocytes are target cells and elliptocytes. Nucleated red cells may be seen if hemorrhage has occurred.

Both the relative and absolute number of reticulocytes may be normal or even slightly increased.

WBC is usually normal but some times eosinophilia may be present. Platelets may be normal, increased or decreased. Thrombocytopenia may occur in severe or long-standing anemia especially if associated with folate deficiency. It has proposed that thrombocytosis is related to iron deficiency caused by chronic blood loss.

**Iron studies:** The serum iron is decreased. It is usually less than  $30\mu\text{g/dl}$  and the TIBC is increased with less than 15% transferrin saturation.

Serum ferritin levels are decreased in all stages of IDA and may be the first indication of a developing iron deficiency. Serum ferritin is an important test to differentiate iron deficiency anemia from other microcytic hypochromic anemia.

Serum ferritin levels are normal in the anemia of chronic disease, and increased in sideroblastic anemia and thalassemia.

**Bone marrow** characterized by decreased M:E ratio, moderate increased of cellularity and mild to moderate erythroid hyperplasia. In erythroid series there is poorly hemoglobinized normoblasts with scanty ragged cytoplasm and erythroid nuclear abnormalities (nuclear fragmentation, multinuclearity). The stains for iron shows absence of hemosiderin in the macrophage and the sideroblasts are markedly reduced or absent.

## **Treatment of Iron Deficiency Anemia**

### **A. Prophylactic**

1. Adequate iron intake for pregnant mothers, a supplement of 30 mg/day should be adequate.
2. Breast-feeding: Has a preventive effect during at least the first 6 months.
3. Prophylactic iron supplementation after the 3rd Month, and earlier in premature by fortified formulas or cereals during infancy.
4. Prophylactic needed after partial gastrectomy.

### **B. Curative**

1. Specific treatment of the causes e.g. as Ancylostoma, or cow- milk protein allergy etc.
2. Oral iron therapy: The best is ferrous sulphate in a dose of 6 mg/kg/day of elemental iron (=30 mg/kg/day ferrous sulphate), in 3 divided doses. Better absorption occurs if given between meals (on empty stomach). It is given for 4-6 weeks after correction of hematological values.
3. Parenteral iron therapy: Iron dextran complex (Imferon). It is not superior to oral iron administration (50 mg iron/ml).

### **Calculation of the dose**

$(\text{Normal Hb} - \text{initial Hb}) \times 1/100 \times \text{blood volume (ml)} \times 3.4 \times 1.5$

Blood volume = 80 ml/kg body wt

3.4 is the amount of iron (mg) in one gram Hb.

1.5 provides extra-iron to replenish iron stores.

4. Packed RBCs: Are given only if there is severe anemia or in presence of infection which may interfere with hematologic response.

If anemic heart failure; a modified exchange transfusion using fresh packed RBCs +/- Furosemide are used.

5. Following correction of anemia, an adequate diet should be instituted.

*A hemorrhage response in the form of reticulocytosis is evidence after 48-72 hours from start of iron therapy. The treatment should continue for 3 months to replenish iron stores.*

## **The Expected effect of Iron Therapy**

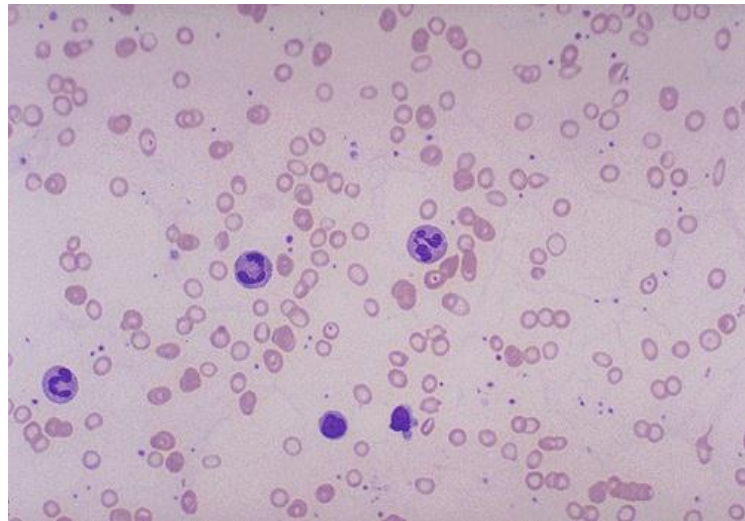
1. Within the first day: Repletion of intracellular iron containing enzymes which leads to increase appetite and improved irritability.
2. Within the first 2 days; bone marrow shows erythroid hyperplasia.



3. At the 3rd day, reticulocytosis appears in peripheral blood, which peak, about the 6th day.
4. From fourth to 30th day; gradual increase of Hb level.
5. From 1-3 months gradual repletion of body iron stores.

#### **Anemia Refractory to Oral Iron**

1. Medication
  - a. Poor preparation (e.g expired drugs)
  - b. Drug interaction
2. Patient
  - a. Poor compliance
  - b. Bleeding continues
  - c. Malabsorption (rare)
3. Physician
  - a. Misdiagnosis
  - b. Consider also:
    - Anemia of chronic disease
    - Thalassemia (minor)
    - Sideroblastic anemia
    - IV Fe



**Figure 3.1: Iron deficiency anemia:** The RBC's are smaller than normal and have an increased zone of central pallor. This is indicative of a hypochromic microcytic anemia. There is increased anisocytosis and poikilocytosis.

## **OTHER CAUSES OF HYPOCHROMIC ANEMIA**

### **CHRONIC LEAD POISONING**

It is a hypochromic microcytic anemia, with coarse basophilic stippling of RBCs.

Diagnosed by increased of free erythrocyte protoporphyrin (FEP) more than 150mg/dl of blood and urinary excretion of large amounts of corpporphirin in urine.

Other manifestations of chronic lead poisoning are usually present as:

Acute abdominal colic associated with constipation, acute lead encephalopathy; with vomiting, ataxia impaired consciousness, convulsions and coma. Peripheral neuropathy (motor) is less common in children than adults.

### **SIDEROBLASTIC ANEMIAS**

These are hypochromic microcytic anemia, caused by defects in iron or heme metabolism. There is elevated serum iron. The bone marrow contains sideroblasts, which are nucleated red blood cells with a perinuclear collar of coarse hemosiderin granules that represent iron-laden mitochondria.

A. X-linked type usually presents in late childhood (splenomegaly is usually present).

B. Vitamin B responsive anemia. Responds to large doses of vitamin B6 (200-500 mg/day)

### **ANEMIA OF CHRONIC DISEASE**

It is seen in patients with infection, cancer, liver disease, inflammatory and rheumatoid disease, renal disease and endocrine disorders (thyroid).

#### **Pathophysiology**

A mild hemolytic component is often present. Red blood cell survival modestly decreased.

Erythropoietin levels are normal or slightly elevated but are inappropriately low for the degree of anemia. Erythropoietin level is low in renal failure.

Iron cannot be removed from its storage pool in hepatocytes and reticuloendothelial cells.

#### **Laboratory Features**

Usually anemia is mild normocytic and normochromic. It may be microcytic and normochromic if the anemia is moderate. May be microcytic and hypochromic if the anemia is severe but rarely  $<90$  g/L.

Bone marrow shows normal or increased iron stores but decreases "normal" sideroblasts.

#### **Iron Indices**

Serum iron is normal or slightly reduced. Total iron binding capacity (transferrin) is normal or slightly reduced. Percent saturation is normal or slightly reduced.

Serum ferritin is normal or increased

### Treatment

Treat the underlying disease. Erythropoietin may normalize the hemoglobin value

Dose of erythropoietin required is lower for patients with renal disease

Only treat patients who can benefit from a higher hemoglobin level.

### REVIEW QUESTIONS

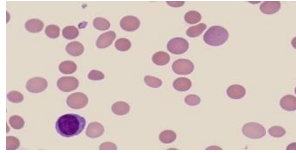
1. What is the primary function of iron
  - a. Molecular stability
  - b. Oxygen transport
  - c. Cellular metabolism
  - d. Cofactor
2. Which of the following influences iron absorption?
  - a. Amount and type of iron in food
  - b. Function of GI mucosa and pancreas
  - c. Erythropoiesis needs and iron stores
  - d. All of the above
3. What is the correct sequence for iron transport?
  - a. Ingestion, conversion to ferrous state in stomach  
reconversion to ferric state in blood stream, transport by  
transferring, incorporation into cells and tissues
  - b. Ingestion, transport by transferring to cells and tissues,  
conversion to ferrous state prior to incorporation into cells  
and tissues.
  - c. A and B are correct
  - d. Non of them
4. In iron deficiency anemia there is characteristically
  - a. An atrophic gastritis
  - b. A low mean corpuscular volume
  - c. A reduced total iron binding capacity
  - d. Megaloblastic changes in the bone marrow
5. What are the two major categories of iron deficiency?
  - a. Defect in globin synthesis and iron incorporation
  - b. Low availability and increased loss of iron
  - c. Defective RBC catabolism and recovery of iron
  - d. Problems with transport and storage of iron
6. Which are characteristic laboratory findings(s) for IDA?
  - a. Increased RDW
  - b. Decreased MCV, MCH, MCHC
  - c. Ovalocytes, elliptocytes, microcytes
  - d. All of the above



7. Which laboratory test results would be most helpful in distinguishing IDA from anemia of chronic disease?
- a. Decreased MCV, MCH, marked poikilocytosis
  - b. Increased MCV, MCH, MCHC, decreased RDW
  - c. Increased RDW and TIBC
  - d. Decreased RDW and TIBC
8. What term refers to the accumulation of excess iron in macrophages?
- a. Sideroblastic anemia
  - b. Hemosiderosis
  - c. Porphyria
  - d. Thalassemia
9. Which of the following would not be seen in sideroblastic conditions?
- a. Increased RDW
  - b. Pappenheimer bodies
  - c. Ringed sideroblasts
  - d. Decreased serum iron
10. What is the characteristic finding in lead poisoning?
- a. Basophilic stippling
  - b. Target cells
  - c. Sideroblasts
  - d. Spherocytes
11. The following statements are correct about the expected effect of iron therapy except:
- a. Within the first 2 days; bone marrow shows erythroid hyperplasia.
  - b. At the 3rd day, Reticulocytosis appears in peripheral blood, which peak, about the 6th day.
  - c. From fourth to 30th day; gradual increase of Hb level.
  - d. From 1-3 weeks gradual repletion of body iron stores.
12. The following statement concern iron deficiency anemia except
- a. stage I :is stage of depletion of iron store
  - b. stage II: impaired erythropoiesis
  - c. stage III: stage of anemia with marked appearing of microcytic RBCs and pathologic indices
  - d. stage IV: The most significant finding is the classic microcytic hypochromic anemia

## HEMOLYTIC ANEMIA

# 4



Hemolytic anemia results from an increase in the rate of red cell destruction. The life span of the normal red cell is 100-120 days; in the hemolytic anemia varying degrees shortens it. The investigation of hemolytic anemia can be challenged and interesting. Many clinicians see it as "all too complicated" and prefer to refer the patient with the question: Has this patient got a hemolytic anemia? As hemolysis is an important diagnosis to establish and may present in a plethora of clinical settings and in many deceptive ways it does behoove most clinicians to have a basic problem solving approach to the initial assessment. If hemolysis is suspected the following questions need to be addressed.

Most hemolysis occurs extravascularly; i.e. in phagocyte cells of the spleen, liver and bone marrow (monocytic-macrophage system). Hemolysis usually stems (1) from intrinsic abnormalities of RBC contents (Hb or enzymes or membrane (permeability structure of lipid content or (2) problems extrinsic to RBCs (serum antibodies (AB), trauma in the circulation or infectious agents). The spleen is usually involved and if splenomegaly results it reduces RBC survival by destroying mildly abnormal RBC or warm AB-coated cells. Severely abnormal RBC or those with cold Abs or complement coating are destroyed with the circulation or in the liver, which (because of its large blood flow) can remove damaged cells efficiently.

The pathways of red blood cell destruction effectively recover heme iron for new red blood cell production. This process is true whether the red blood cells break down in circulation (intravascular destruction). Destruction of senescent cells is largely limited to extravascular pathway. Red blood cells are phagocytized by the reticuloendothelial cells, the membrane is disrupted, and hemoglobin is broken down by lysozymal enzymes. The iron recovered from heme is then stored or transported back to the marrow for new red blood cell production. Amino acids are also recovered. At the same time, the protoporphyrin ring is metabolised to the tetrapyrrol (bilirubin) with the release of carbon monoxide. The bilirubin is subsequently transported to liver where it is conjugated and excreted into bile.

Intravascular hemolysis is uncommon; it results in hemoglobinuria when the Hb released into plasma exceeds the Hb-binding capacity of plasma haptoglobin. Hb is reabsorbed into renal tubular cells when Fe is converted to hemosiderin, part of which is assimilated for reutilization and part of which reaches the urine when the tubular cells sloughs. Identification of hemosiderinuria in a fresh urine specimen provides clear evidence of an intravascular hemolytic process.

**Table 4.1 Diagnostic criteria of hemolysis**

**1. GENERAL EVIDENCE OF HEMOLYSIS**

- Jaundice and hyperbilirubinemia
- Reduced plasma haptoglobin and hemopexin
- Increased plasma lactate dehydrogenase

**2. EVIDENCE OF INTRAVASCULAR HEMOLYSIS**

- Hemoglobinemia
- Hemosiderinuria
- Hemoglobinuria
- Methemalbuminemia

**3. EVIDENCE OF COMPENSATORY ERYTHROID HYPERPLASIA**

- Reticulocytosis
- Macrocytosis and polychromasia
- Erythroid hyperplasia of the bone marrow
- Radiological changes of the skull and tubular bone

**4. EVIDENCE OF DAMAGE TO THE RED CELLS**

- Spherocytosis and increased red cell fragility
- Fragmentation of red cells
- Heinz bodies

**5. DEMONSTRATION OF SHORTENED RED CELL LIFE SPAN**

- Reticulocytosis and hyperbilirubinemia are the main criteria suggesting overt hemolytic anemia.

**6. SUGGESTIVE CLINICAL FEATURES OF HEMOLYSIS?**

Family history

Past history e.g., jaundice, gallstone, leg ulcers.

Recent history e.g., jaundice, dark urine, pains, Raynaud's phenomenon, shivers and sweats, pyrexia, blood transfusion, toxin exposure, envenomation, burns.

## **Hyperbilirubinemia**

If liver and biliary functions are normal an unconjugated hyperbilirubinemia occurs in hemolysis and the only real differential diagnosis is that of Gilbert's syndrome which is usually a diagnosis of exclusion. The standard teaching that a conjugated hyperbilirubinemia excludes hemolysis is incorrect. Firstly, the techniques used for measuring conjugated bilirubin are indirect and not truly accurate. Normal person should not have any detectable conjugated bilirubin present, but up to one third of the bilirubin may appear conjugated by the methods used in most laboratories.

There are several clinical situations in which there may be a combination of hemolysis in conjugation with impaired hepatobiliary handling of conjugated bilirubin. The system may thus be stressed by hemolysis with hepatic conjugation proceeding normally, but a build up occurring at the excretory level. Excluding the obvious biliary obstruction from gallstone (possibly pigment stones in a chronic hemolytic anemia) is the commonest



defect seen in seriously ill patients (who are high-risk candidates for hemolysis secondary to blood transfusion, drugs, infections etc) at the energy-requiring level of the active transport of conjugated bilirubin from the hepatocyte into the biliary canaliculus. Many patients with acute hemolysis are also likely to have impaired bilirubin transport due to the effects of shock or sepsis. Under these circumstances bilirubin from hemolysis will be rapidly conjugated but will have delayed excretion and manifest as a conjugated hyperbilirubinemia.

### Reticulocytosis

Reticulocytes are usually expressed as a percentage of the red cells. In normal subjects the reticulocyte count varies from 0.2-2%. In hemolytic anemia it usually ranges from 5-20% but occasionally raises too much higher values e.g. 50-70% or even more. In acute hemolysis there may not have been time for the bone marrow to respond, as some days are usually necessary for erythropoiesis to increase to a point where the reticulocytosis is manifest in the peripheral blood. As long as the hematocrit and level of erythropoietin stimulation are normal, the observed reticulocyte percentage may also be considered of production (normal reticulocyte production index=1). With a lower than normal hematocrit level, however, the reticulocyte count must first be corrected to a hematocrit of 45 before calculating the reticulocyte production index (RPI). A further correction is required when erythropoietic stimulation results in premature delivery of marrow reticulocytes to circulation ("shift reticulocytes"), since these younger cells require 2 to 3 days to lose their reticulum. With increasing anemia, there is a progressive lengthening of the reticulocyte maturation.

**Table 4.2 : Maturation time correction**

Hematocrit	Reticulocyte maturation time
0.45	1.0
0.35	1.5
0.25	2.0

### Corrected Reticulocyte Count:

*Reticulocyte count (%) X Patient packed cell volume/Normal Hematocrit based on age and sex =%*

**The reticulocyte production index may be calculated as follows:**

$$\frac{\text{Reti count (\%)} \times \text{Patient's Hematocrit (Hct)}}{45 \text{ (Normal Hct)}} \div \text{Reticulocyte Maturation Time}$$

Example: A patient has a reticulocyte count of 9%, a hematocrit of 25%, and easily visible "shift" red cells (Polychromasia) on the peripheral smear.

$$\text{RPI} = \frac{9\% \times 25}{45} \div 2.0 = 2.5 \%$$

### **Elevated Lactic Dehydrogenase (LDH), or Isoenzyme Hydroxybutric Dehydrogenase (HBDH)**

Lactic dehydrogenase is a cytoplasmic enzyme and can be helpful in determining the presence of hemolysis. An isolated marked elevation of LDH with other "profile" enzymes remaining normal (AST, ALT, CPK, ALP) is highly suggestive of red cell destruction (hemolysis or ineffective erythropoiesis). Red cells do not contain mitochondria so the levels of other "standard" enzymes for tissue damage (e.g., ALT and AST) which are mitochondrial in origin are normal or only marginally elevated.

### **Haptoglobins**

This family of hemoglobin binding plasma proteins will be reduced if significant hemolysis is occurring. Haptoglobins are acute phase reactants and may be elevated in infection and inflammation. In contrast they may be rapidly reduced by blood transfusion as a result of nonsurviving-stored red cells. If they are present or increased significant hemolysis can be interpreted with caution.

The normal range is 30-200 mg/100 ml. It is estimated either chemically or by electrophoresis.

### **Hemosiderinuria**

A Week after an episode of intravascular hemolysis, or in-patients with chronic intravascular hemolysis, examination of the urine with a Prussian blue stain for iron may confirm the presence of hemosiderinuria. After the kidney filters the dimers of hemoglobin they are resorbed by the proximal tubules and metabolised. The resultant iron can be found in the tubular cells shed into the urine.

### **Blood Film**

Whatever it was the cause of hemolysis. The end result is rupture and destruction of the red cell. This rupture involves membrane damage, which need not be complete from the outset, so degrees of development of red cell damage may be observed in the circulating red cells.

### **Methods Used For Diagnosis of Hemolytic Anemia**

1. High peripheral reticulocytes more than 2% (usually >10%) + nucleated RBCs in the peripheral.
2. Bone marrow aspiration: Increased erythroid tissue; with low Myeloid/Erythroid ratio. Normally it is 2:1 to 4:1
3. Hyperbilirubinemia (indirect).
4. High serum irons and low iron-binding capacity.
5. Low red blood cell life span (Normal is 120 days).
6. High urobilinogen in urine (Normal level is 2-5 mg/d and in stool is 30-300 mg/d)
7. Hemoglobinuria in acute hemolysis.
8. Serum haptoglobin and hemopexin are low.

9. High Co in blood and expired air due to degradation of Hb to bilirubin.

10. X-Ray picture in chronic hemolytic anemia:

A. Skull-wide diploic space and hair on end appearance.

B. Long and short long bones: wide medullary space and thin cortex

- Mosaic appearance of bone

- Pathologic fractures may be present

C. Cholecystography; may show bile stones

D. Chest and heart; cardiomegaly and heart failure may be present.

### **What is the precise diagnosis?**

#### **1. If a hereditary hemolytic anemia is suspected**

- Osmotic fragility determination (\*).
- Autohemolysis test +/- the addition of glucose (\*\*).
- Screening test for red cell G6PD deficiency.
- Red cell pyruvic kinase assay.
- Assay of other red cell enzymes involving in glycolysis.
- Sickling preparation for Hbs
- Electrophoresis for abnormal Hb estimation
  - HbF → in beta thalassemia and sickle cell anemia
  - HbS → in sickle cell anemia
  - HbC → in Target cell anemia

#### **2. If an autoimmune acquired hemolytic anemia is suspected**

- Direct antiglobulin test using anti-Ig and anticomplement sera
- Test for the auto-bodies in the patient serum
- Titration of cold agglutinins
- Electrophoresis of serum proteins

#### **3. If hemolytic anemia is suspected of being drug induced**

- Screening test for red cell G6PD
- Staining for Heinz bodies

#### **4. In all instance of hemolytic anemia of obscure type (and in all cases of aplastic anemia)**

- Acidified serum test (Heinz test) for paroxysmal nocturnal hemoglobinuria.
- Cold antibody lysis test.

**\*Osmotic Fragility Test** is increased in most hemolytic anemia (spherocytes). In congenital spherocytosis the initial hemolysis occurs in 0.73 saline solutions and is complete in 0.40 saline solutions. Decreased osmotic fragility is noted in thalassemia, sickle cell disease, HbC, iron deficiency anemia. In thalassemia occurs in 0.50 and is complete in 0.07 saline solutions.



**\*\*Autohemolysis:** Incubation of normal sterile defibrinated or oxalated blood at 37°C in their own serum with or without added glucose shows only spontaneous hemolysis during the first 24-48h, after which time rapid autohemolysis takes place. This acceleration is due to several factors among which spherocytosis, action of antibodies and action of chemicals can be mentioned. The rate of hemolysis can be slowed by the addition of glucose, mannose.

Normal values after 48 hours incubation are:

Without added glucose, 0.2-2% haemolysis

With added glucose, 0.01-0.9%

## **Etiological Classification of Hemolytic Anemia**

### **A. Due to intracorporeal mechanism**

#### **I. Congenital**

##### **1. Membrane defect**

H. Spherocytosis, H. elliptocytosis, H. hydrocytosis.

##### **2. Hemoglobin defect**

a. Hemoglobinopathies; due to abnormal molecular structure: Sickle cell anemia,

HbC, HbE, HbD etc

b. Rate of synthesis: e.g. B-Thalassemia major, alpha Thalassemia.

c. Double heterogeneous disorder: Sickle cell B-Thalassemia

##### **3. Enzyme defect**

a. Non-spherocytic congenital hemolytic anemia

Pyruvate kinase deficiency and G.6.P.D deficiency

b. Drug-induced hemolytic anemia and favism

### **II. Paroxysmal nocturnal hemoglobinuria**

#### **B. Due to extracorporeal mechanism**

##### **I. Immune mechanisms**

Autoimmune acquired hemolytic anemia

Hemolytic disease of the newborn

Incompatible blood transfusion

Drug induced hemolytic anemia

##### **II. Non-immune mechanism**

Mechanical hemolytic anemia

March hemoglobinuria

Cardiac hemolytic anemia

Microangiopathic hemolytic anemia.

## REVIEW QUESTIONS

1. In the absence of blood loss, the laboratory result most useful in the initial diagnosis of a hemolytic anemia is a (an)
  - a. Increased reticulocytic count
  - b. Increased total bilirubin level
  - c. Decreased Hb and Hct
  - d. Increased LDH
2. The laboratory test that is abnormal because of intravascular hemolysis, but usually normal with increased extravascular hemolysis
  - a. Fecal urobilinogen
  - b. Urine urobilinogen
  - c. Reticulocyte count
  - d. Plasma hemoglobin
3. The substance that is present in the urine in increased amounts if extravascular hemolysis is increased but there is no intravascular hemolysis is increased but there is no intravascular hemolysis
  - a. Methemoglobin
  - b. Urobilinogen
  - c. Hemoglobin
  - d. Hemosiderin
4. A decrease in the concentration of -----in plasma is the earliest indicator of a decrease rate of hemolysis.
  - a. Haptoglobin
  - b. Lactate dehydrogenase
  - c. Serum haptoglobin
  - d. Urine free hemoglobin
5. If an intravascular hemolysis is suspected, but hemoglobinemia is not detected visually or spectrophotometrically, the first test that could be recommended that may confirm the suspicion is
  - a. Serum heopexin
  - b. Serum Lacate dehydrogenase
  - c. Serum Haptoglobin
  - d. Urine free hemoglobin
6. Hemolytic anemia is not indicated by a (an)
  - a. Positive urine hemosiderine
  - b. Positive fecal occult blood
  - c. Increased plasma unconjugated bilirubin
  - d. Decreased serum haptoglobin
7. If a male patient has a reticulocyte count of 6% and a packed cell volume of 0.45 L/L, what is his RPI%?
  - a. 1.5%
  - b. 3%
  - c. 4.5%
  - d. 6%

8. If a male patient has a reticulocyte count of 5% and a packed cell volume of 0.45 L/L. What is his corrected reticulocyte count?

- a. 2.5 %
- b. 4.5 %
- c. 5.0 %
- d. 10 %

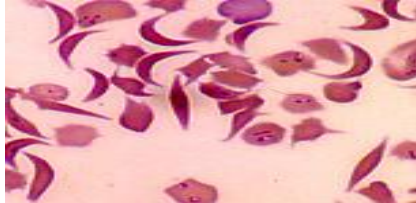
9. The following statements about hemolytic anemia are true except:

- a. Is often accompanied by an increase in serum unconjugated bilirubin
- b. Is usually accompanied by increased urinary bilirubin
- c. Is predominantly extravascular in hereditary spherocytosis
- d. Can lead to kernicterus in the neonate



## SICKLE CELL HEMOGLOBINOPATHIES

# 5



The term sickle cell disease is used generically to describe a group of genetic disorders characterized by the production of the abnormal hemoglobin S (HbS). Sickle cell anemia (SCA) is the most common type of sickle cell disease and represents the homozygous form, in which the individual inherits a double dose of the abnormal gene, which codes for hemoglobin S. This type of hemoglobin differs from normal hemoglobin by the single amino acid substitution of valine for glutamic acid in the sixth position from the NH<sub>2</sub> terminal end of the  $\beta$  chain. The structural formula for sickle cell anemia (HbSS) is  $\alpha\beta^2$ .

### Pathophysiology

1. When oxygenated, HbS is fully soluble. Sickling occurs when oxygen decreases at the tissue level. When oxygen is released from the Hb molecules, a conformational change occurs, which results in polymerisation of the Hb molecule and leads to the formation of tactoids or crystals, which cause the cell to become rigid.
2. The distorted and rigid sickle cells impede blood flow to tissues and organs, resulting in tissue death, organ infarction, and pain.
3. Repeated of sickle-unsickle cycles lead to loss of fragments of red cell membrane, and the cells become spherocytic and fragile. They are removed prematurely by the reticulo-endothelial system, and to a lesser extent destroyed in the circulation resulting in both extravascular and intravascular hemolysis. The bone marrow responds by increasing erythropoiesis. As a result, the marrow spaces widen and the bone cortex thins.

The amount of HbS in the red cell is clearly of great importance. The cells of a patient with sickle cell trait which contain less than 50% HbS are less likely to sickle at a particular level of deoxygenation than the cells of a patient with homozygous sickle cell disease which contain 100% HbS

### Sickle Cell Trait

Patients with one normal  $\beta$ -globin gene and one  $\beta^s$  -gene are said to have sickle cell trait. This condition is by far the most common of the sickle cell variants; it is asymptomatic carrier state for HbS. HbS comprises 38-45% of the total hemoglobin, the rest being HbA, HbA<sub>2</sub> and HbF. The cells do not contain sufficient HbS to undergo sickling at the lowest oxygen tension

normally occurring in the body and the red cell lifespan is normal. In the stained blood film, no sickle cells are present and the red cells appear normal. The MCV and MCH are normal. However sickling can readily be demonstrated by the sickle test, and the hemoglobin solubility test is positive. The sickle cell trait does not cause anemia, and in general is asymptomatic. If anemia is present, other causes, e.g. iron deficiency, should be sought.

### **Homozygous Sickle Cell Disease**

The worst of the sickle cell diadease is sickle cell anemia. Like othe hemoglobinopathies, it is an inherited disease. I s is trabsnitted in an autosomal recessive manner, occurring in persons who have inherited two  $\beta^s$ -globin genes. The patient receives one HbS from each parent, both of whom show sickle cell trait. The probability for each child such unions to have normal hemoglobin only, sickle cell trait or homozygous disease are 25%, 50% and 25%. In these persons, hemoglobin S accounts for more than 90% of the hemoglobin in the red cell.

In sickle cell disease, the red cells contain sufficient HbS for sickling to be produced in vivo by the reduction of oxygen tension that occurs in capillaries.

In vivo sickling is responsible for the clinical manifestations of the disease. These are chronic hemolytic anemia, organ damage and episodes of pain.

**Clinical manifestations** fall into two categories: those caused by the anemia and those attributable to occlusions of the microvasculature.

The diagnosis is usually made in childhood (before two years). Symptoms are infrequent in first six months (High HbF protecting the RBC from sickling). Many children died in the first 7 years. Bacterial infection is the most common cause of mortality and morbidity. (e.g. Pneumococcal meningitis). Hand foot syndrome is caused by microinfarction of the medulla of the carpal and tarsal bones. This syndrome manifested by hand and feet tender, swollen and febrile.

**Spleen** is palpabe between six months and 8 years. Splenic sequestration syndrome developed due to sudden pooling of blood within the spleen, often with acute hypovolemia and shock. The spleen enlarges rapidly and death may occur.

Blood film: Howell-Jolly bodies and target cell found in splenectomized patients.

Repeated episodes of infarctions eventually lead to atrophy and autosplenectomy; by eight years of age, the spleen is no longer palpable and its function is permanently impaired.

**In Adult** all patients are anemic (many of them adapt to anemia).

Sickle cell crises are characteristic features of disease and are responsible for morbidity.

A. Vaso-occlusive crises :( Occlusion--Ischemia--Infarction) consist of sudden attacks of bone pain usually in the limbs, joints, back and chest or of abdominal pain.

B. Aplastic crises: occur when there is sudden cessation of marrow erythropoiesis related to infection with human parvovirus. Hemolysis continues and the red cell mass rapidly diminishes to life-threatening

levels. Significant reductions are in erythrocyte count, Hb, Hct, reticulocyte count and bone marrow erythroblasts.

C. Infectious crises: Children with sickle cell anemia have an increased susceptibility to potentially life-threatening bacterial infections, including sepsis and meningitis caused by streptococcus pneumoniae and Haemophilus influenzae. The relative risk of sickle cell anemia patients compared with that of normal individuals for pneumococcal H. influenzae and all bacterial meningitis is 579:1, 116:1, and 309:1, respectively. These patients also are susceptible to bacterial pneumonia, osteomyelitis (salmonella and staphylococcus) and urinary tract infections (Escherichia coli and klebsiella). Increased susceptibility also is seen for shigella and Mycoplasma pneumoniae. Bacterial infection is the most common reason for hospitalization of pediatric patients with sickle cell anemia and often leads to the diagnosis. Serious bacterial infections are seen in one third of children with sickle cell anemia before 4 years of age and a primary cause of death these patients.

#### **Other Features**

Conjunctival icterus is common and liver enlarged and some times is tender due to infarction. Cholelithiasis is common.

Cardiac enlargement and systolic ejection murmur/thrills.

Progressive loss of renal function occurs in many patients

Chronic leg ulcers are common.

Bone: Osteomyelitis due to salmonella and avascular necrosis of the femoral or humeral head.

#### **Blood Picture**

Anemia: Hb of 6-9 g/dl is usual but they may be lower and an occasional patient has a normal value. The anemia is due reduction of red cell life span and in some patient due to hypersplenism (red cell destruction). The anemia is usually normochromic normocytic with a normal MCV and MCH.

Blood Smear: Anisocytosis, Poikilocytosis with elongated, rounded, sharper to typical sickle cells. Oval cells are common and occasional target cells and Howell-Jolly bodies are present. Nucleated RBC may be present (polychromatic cells). Leukocytes increased with shift to left.

Reticulocyte is increased (10-20%). The ESR is low even with marked anemia as the abnormal shape of the sickle cells prevents rouleaux formation. Serum folate is subnormal and the red cell folate is low. Serum haptoglobin and hemopexin are decreased and serum bilirubin is moderately increased

#### **Sickle Cell Preparation**

The metabisulfate (reducing agent) is added to a suspension of whole red cells in saline. Cells containing any quantity of hemoglobin S - The test is simple to perform and detects even those from patients with sickle cell trait will sickle under these conditions.



### Hemoglobin Solubility Test

The basis of these tests is the relative insolubility of reduced HbS in concentrated phosphate buffer. In practice, the hemoglobin is added to a solution of sodium dithionite, a reducing agent, in phosphate buffer. If HbS is present, the solution becomes turbid. Homozygotes and heterozygotes for the sickling gene are detected.

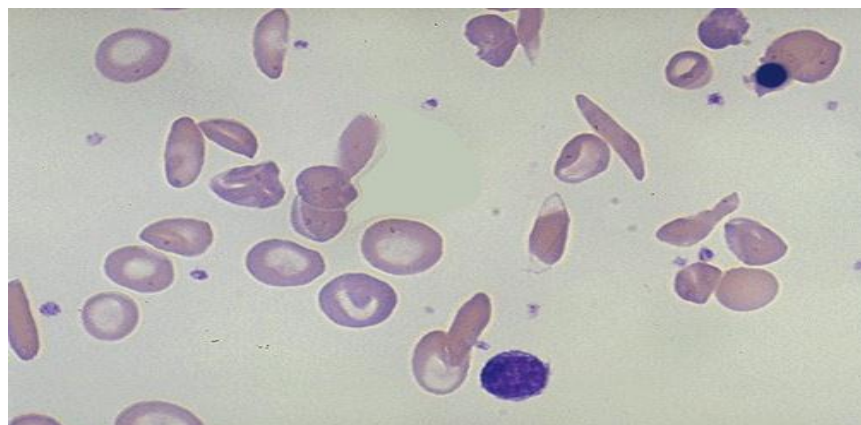
Currently, prenatal screening with recombinant DNA technology is done; the availability of the polymerase chain reaction has remarkably improved sensitivity of prenatal diagnosis.

### Hemoglobin Electrophoresis

It is the mandatory for precise diagnosis of the sickle hemoglobinopathies. HbS may be demonstrated by electrophoresis on cellulose acetate at PH 8.6 in a position between HbA and HbA2 or agar gel electrophoresis using citrate buffer done at PH 6.0.

**Table 5.1: HB-electrophoresis of sickle hemoglobinopathies**

Disorder	A	A2	F	S
Sickle cell trait(AS)	55-70	2-4	N	38-45%
Sickle cell disease (SS)	0	2-5	1-20	75-95%
Sickle cell Beta-Thalassemia	10-30	4-8	2-10	60-85
S-B <sup>+</sup>	0	4-8	5-30	70-90
S-B <sup>0</sup>				
Sickle cell Hb-C disease	0	35-50	1-5	50-65
Sickle cell Hb-D disease	0	N	1-5	95 (S+D)
S.C.trait- $\alpha$ thalassemia trait	65-75	N	N	20-30



**Figure 5.1: Peripheral blood smear shows marked poikilocytosis and anisocytosis with numerous sickled erythrocytes**

## Treatment of Sickle Cell Anemia

With advances in the diagnosis, treatment and prevention of complication, the life expectancy of individuals with sickle cell disease has improved. There is an 85% chance that infants with SCA will survive to age 20 or more.

The principal causes of death in infants with SCA include overwhelming infections with *S. pneumonia*, cardiovascular accidents, and acute splenic sequestration crises. The treatment program includes the following:

1. Repeated packed RBCs transfusion: May be required for acute situation and for prevention of certain complication of SCA.
2. Iron chelation agents: May be needed to prevent hemosiderosis (desferal + SHAM).
3. Vigorous treatment of complicating bacterial infections.
4. Polyvalent pneumococcal vaccine and *Hemophilus influenza* type B vaccine are better given after 2 years age.
5. Prophylactic long acting penicillin may be given every 4 weeks, which reduces both morbidity and mortality from pneumococcus infection in SCA in infants.
6. Treatment of crises:
  - a. Pain crises: Correct the factors: infection, hypoxia, dehydration
    - Give packed RBC
    - Analgesics as codeine or phenothiazine
    - " Avoid addiction"
    - Avoid dehydration and acidosis
  - b. Aplastic crises: Give packed RBCs
  - c. Sequestration crises: Plasma expanders
- Blood transfusion
- Emergency splenectomy may be indicated
  - d. Megaloblastic crises: folic acid 1mg/day is preventive.
7. Hydroxyuria therapy appear to provide an increment in Hb content and a decrease in incidence of pain crisis.

Why this drug works in SCA is not completely understood, but it is believed to include the production of HbF in RBCs. Increased levels of HbF in RBCs prevent the sickling, therefore preventing vasoocclusion and painful crises. Hydroxyuria, however is a cytotoxic agent and has the potential to cause life threatening cytopenia. Therefore, SCA patients using this drug must be carefully evaluated and monitored.
8. 5-(2-formyl-3hydroxyl) pentamolic acid and Bepridil is under study. Also clotimazole, magnesium, nitric oxide are under evaluation.

## HEMOGLOBIN C SYNDROME

HbC is caused by substitution of lysine for glutamic acid in the sixth position from the N-terminal end of the  $\beta$ -hemoglobin chain.

Hemoglobin C Trait (HbAC) patients with this syndrome are asymptomatic and not anemic. The peripheral blood smear shows

increased numbers of target cells and Hb-electrophoresis reveals about 40% hemoglobin C migrating faster than HbS but slower than HbA. Hemoglobin C disease (HbCC) is characterized by a mild hemolytic anemia associated with splenomegaly and by peripheral blood smear showing many target cells and some microspherocytes. Hemoglobin C crystals may appear after slow drying of a peripheral blood smear and may account for the marked targeting, with puddling and then crystallization of hemoglobin in the center of these cells.

## HEMOGLOBIN D (HB D) DISEASE AND TRAIT

Result from the inheritance of one gene for HbS from one parent and one gene for HbC from the other parent. HbD has several variants. Both homozygous ( $\alpha 2\beta 2^{121 \text{ Glu Gln}}$ ) and the heterozygous ( $\alpha 2\beta 1\beta 1^{121 \text{ Glu Gln}}$ ) states are asymptomatic.

The peripheral blood smear is undermarkable, except for a few target cells. Hemoglobin migrate electrophoretically to the same position as HbS and HbC at Alkaline PH but migrates with HbA at acid PH. HbD is non-sickling soluble hemoglobin.

## HEMOGLOBIN E (HBE)

HbE results from the substitution of lysine for glutamic acid in the beta chain of Hb. It occurs with greatest frequency in Burma, Thailand, Cambodia, Laos, Malaysia and Indonesia. The homozygous state ( $\alpha 2\beta 2^{6 \text{ Glu Lys}}$ ) presents with little anemia; target cells and microcytic hypochromic red cells. HbE trait ( $\alpha 2\beta 1\beta 1^{26 \text{ Glu-Lys}}$ ) is asymptomatic clinically. There is microcytosis, target cells and approximately 70% HbA and 30% HbE and A2 on routine electrophoresis.

## HEMOGLOBIN Oarab (HbOarab) DISEASE AND TRAIT

HbOarab is rare hemoglobin variant that occurs infrequently in blacks Arab and Sudanese population. Homozygous HbOarab ( $\alpha 2\beta 2^{121 \text{ Glu Lys}}$ ) exhibits a mild hemolytic anemia with slight splenomegaly and target cells on the peripheral blood smear. This hemoglobin migrates electrophoretically with HbC, HbE and HbA2 at alkaline PH. In the heterozygous state of HbOarab ( $\alpha 2\beta 1\beta 1^{121 \text{ Glu-Lys}}$ ) the patient is asymptomatic.

## HEMOGLOBIN S WITH OTHER ABNORMAL HEMOGLOBIN

### HEMOGLOBIN SB THALASSEMIA

Because of the increased frequency of both HbS and B-thalassemia genes in similar population groups, inheritance of both defects is relatively common. Clinically, the disorder produces symptoms of moderate anemia and many signs of sickle cell anemia, which are usually less frequent and less severe.



Laboratory findings are mild to moderate microcytic anemia, some sickled RBCs on stained blood smear, and reticulocytosis. The HbA<sub>2</sub> is > 3% HbS predominates on electrophoresis and HbA is decreased or absent. HbF increase is variable.

Treatment: Like sickle cell anemia.

### **HEMOGLOBIN SC DISEASE (HbSC)**

HbSC is of intermediate severity between hemoglobin SS disease and hemoglobin CC disease. Patients may have a mild anemia, with hemoglobin ranging from 10 to 13 gm/dl and a reticulocytosis of 3 to 10%. In the peripheral smear, target cells are more prominent than sickled cells. Hemoglobin electrophoresis shows equal amounts of hemoglobin S and C. The clinical course varies from asymptomatic to severe.

### **HEMOGLOBIN SD DISEASE (HbSD)**

HbSD is the combination of HbS and HbD. Because these hemoglobins migrate together at alkaline pH, the electrophoretic pattern is similar to that of SCA.

### **HEMOGLOBIN SO<sub>Arab</sub> (HbSO<sub>Arab</sub>)**

The combination of HbS and HbO<sub>Arab</sub> can have a clinical presentation that is similar in severity to that of SCA. The anemia is severe, with typical sickle cells seen on the peripheral blood smear. This condition might initially be confused with HbSC on routine electrophoresis; however differentiation can be made with acid electrophoresis.

### **REVIEW QUESTIONS**

1. Abnormal hemoglobin are most often caused by
  - a. Amino acid substitutions
  - b. Amino acid deletion
  - c. Globin chain elongation
  - d. Globin chain fusion
2. Which one of the followings is not a characteristic of hemoglobinopathies?
  - a. Conditions in which abnormal hemoglobins are synthesized
  - b. Result from inherited abnormalities or genetic mutation
  - c. All are manifested in clinically significant conditions
  - d. Result in a defect instructional integrity of function of the hemoglobin molecule.

3. The most common cause of hemoglobinopathies is an abnormality in the ----- globin chain
- $\alpha$
  - $\beta$
  - $\gamma$
  - $\delta$
4. What factors contribute to the sickling of RBCs?
- Increase in PH and oxygenation
  - Decrease in PH and oxygenation and dehydration
  - Increase in PH and decrease in oxygenation
  - Decrease in dehydration and increase in PH and oxygenation
5. What are the therapeutic goals in the treatment of sickle cell anemia?
- Decrease microvascular entrapment of sickle cells or change the volume of RBCs
  - Modify oxygen affinity or solubility of sickle hemoglobin
  - Increase production of fetal hemoglobin
  - All of the above
6. Laboratory values that could be found in a patient with sickle cell anemia (HbSS) disease includes all of the following except:
- 40% HbS on cellulose acetate electrophoresis
  - 7% HbA2 on cellulose acetate electrophoresis
  - Normocytic, normochromic anemia
  - Hemoglobin 6.0 g/dl
7. Cellulose acetate hemoglobin electrophoresis is run at a (an)
- Alkaline PH
  - Acid PH
  - PH gradient
  - Alkaline and acid PH
8. The condition (s) associated with increased levels of HbF is (are)
- Infancy
  - Hemoglobinopathies
  - Thalassemia
  - All of the above
9. Two HbS that migrates together on cellulose acetate electrophoresis at alkaline PH are
- A1 and A2
  - A1 and E
  - S and C
  - S and D

The thalassemias are a heterogenous group of disorders with a genetically determined reduction in the rate of synthesis of one or more types of the normal hemoglobin polypeptide chain. This results in a decrease in the amount of the hemoglobin involving the affected chain.

## Classification

There are two main groups of thalassemia:

1. Alpha thalassemia, affecting the synthesis of alpha chain
2. Beta Thalassemia, affecting the synthesis of Beta chain

1. Alpha thalassemia:

Single gene deletions give rise to the so-called silent carrier state and the person is normal, both clinically and hematologically.

**Table 6.1:  $\alpha$ -Thalassemia syndrome : Phenotype , Genotype**

Phenotype	Genotype	Abbreviation
Normal	Normal	$\alpha \alpha$
Silent carrier of $\alpha$ thalassemia	Heterozygous $\alpha^0$	$\alpha \alpha^+$
$\alpha$ -Thalassemia trait	Heterozygous $\alpha^0$	$\alpha \alpha^0$
$\alpha$ -Thalassemia trait	Homozygous $\alpha^+$	$\alpha^+ \alpha^+$
Hemoglobin H disease	Copmpound heterozygous $\alpha^+ \alpha^0$	$\alpha^0 \alpha^0$
Hemoglobin Bart's hydrops fetals	Homozygous $\alpha^0 \alpha^0$	$\alpha^0 \alpha^0$

## HbH DISEASE

HbH ( $\beta_4$ ) disease usually results from coinheritance of  $\alpha^+$  and  $\alpha^0$  thalassemia alleles ( $--/-\alpha$ ), with  $\alpha$  chain production only 25% to 39% of normal, but there are non-deletional forms ( $--/\alpha\alpha$ ) as well. It is characterized by a variable degree of anemia with splenomegaly and a typical thalassemia blood picture.

On the peripheral blood film, red cells are microcytic, hypochromic with anisopoikilocytosis. The RDW is increased. Almost all red cells have HbH inclusion, which are visible microscopically when erythrocytes are incubated and supravitaly stained with brilliant crysyl blue (BCB). HbH inclusions generally occur in multiples and cover the cell surface, producing, a golf-ball like appearance.

Hb electrophoresis (alkaline PH) in affected neonates shows about 25% to 40% Hb Barts ( $\gamma_4$ ), but as  $\beta$ -chain synthesis replaces  $\gamma$  during the first few months of life, Hb Barts is gradually replaced by HbH ( $\beta_4$ )

Most individuals with HbH disease require no therapy; their growth, development and life expectancy are usually normal.

Hb Bart's (4 gamma chain) hydrops syndrome results from deletion of all four alpha genes and is a common cause of stillbirth.



## THE BETA THALASSEMIA

The genetic mutation of beta thalassemia leads to a decreased rate of beta chain synthesis and consequently a reduction in the amount of normal HbA in the red cells. A microcytic hypochromic anemia is results.

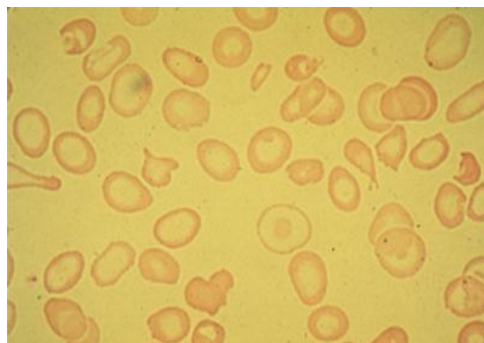
On the basis of the extent of reduction of Beta chain synthesis, two main types of beta thalassemia are recognized.

- B<sup>+</sup> thalassemia is characterized by incomplete suppression.
  - B<sup>0</sup> thalassemia is characterized by complete absence of beta chain synthesis.
- Clinically, beta thalassemia occurs in two forms. Beta thalassemia major or Cooley's anemia is usually a severe illness characterized by the major or total suppression of chain synthesis and is the homozygous form of the disease.

Beta thalassemia minor, or trait, is a mild and some times asymptomatic condition and represents the heterozygous form.

**Table 6.2: The beta thalassemia and related syndromes**

Disorder	HbA%	HbA2%	HbF%
B-thalassemia minor	90-95	3.5-7	1.5
B-thalassemia Intermedia			
β-Thalassemia <sup>+</sup>	Present	5.4 – 10%	30-73%
β-Thalassemia	Present	>3.2	1.5-12
β-Delta-Thalas. Intermediate	0	0	100
β-δ Thala. Minor	80-95	1-3.5	5-20
Beta thalassemia major			
B Thalassemia <sup>+</sup>	10-90	1.5-4	10-90
B Thalassemia <sup>0</sup>	0	1.5-4	98
β-δ Thala Major	Present	0.6 3.4	>75%
Thalassemia minimum	60-85	1-2	15-35
Hereditary persist. of fetal Hb (HPFH)	60-85	1-2	15-35



**Figure: 6.1**

**Thalassemia major:**

The blood smear show hypochromic microcytic anemia in which targets cells, tear drops, and fragments are frequent.

## BETA THALASSEMIA MINOR (TRAIT)

It is a heterozygous state for the Beta thalassemia gene.

### Clinical Features

Mild disorder with little or no anemia, there are no symptoms, and a normal life expectancy. The spleen may be palpable.

The condition is commonly not diagnosed until adolescence or adult life, and may be detected in a routine hematological screening examination. It is often first diagnosed in pregnancy.

### Laboratory Finding

**Hemoglobin** is normal or mild reduced. RBCs are normal in spite of mild anemia and may be increased. MCV and MCH are reduced. MCHC is usually marginally reduced or normal.

**Blood smear** characterized by mild anisocytosis, microcytosis and hypochromia with variable numbers of target and stippled cells.

**The osmotic fragility test** shows an increased resistance to hemolysis even when anemia is absent.

**The serum bilirubin** is normal or slightly raised. Hb A2 is increased HbF increased in 50% (Absence of HbF not exclude the diagnosis)

The main problem in the diagnosis is the differentiation of beta thalassemia minor from iron deficiency anemia. Clinical features are not helpful.

In the usual case of thalassemia minor, red cell microcytosis hypochromia and reduction in MCV are relatively marked considering the mild or absent anemia. This is not the case in iron deficiency in which there is closer relation between morphological abnormalities and the degree of anemia.

Estimation of iron, ferritin, transferrin, HbA2 and HbF are helpful for diagnosis.

## Thalassemia Intermedia

Thalassemia intermedia are the clinical appellation for patients with two  $\beta$ -thalassemia genes but whose phenotype is less severe than thalassemia major. The most commonly associated genotypes are  $\beta^+/\beta^+$  or  $\beta^+/\beta^0$ , with a relatively high  $\beta$ -globin producing  $\beta^+$  mutation. The disease is extremely heterogenous at the genotypic level. Coinheritance of  $\alpha$ -thalassemia or hereditary persistence of fetal hemoglobin reduces disease severity and may transform what would have been a thalassemia major phenotype into thalassemia intermedia. Affected persons are typically asymptomatic, but the spectrum of disability is broad. The most frequent complications are hypersplenism, gallstones, and ankle ulcers. Patients with thalassemia intermedia should be given folate supplementation and periodic red blood cell transfusions and be monitored for iron overload.

## Beta Thalassemia Major

It is the homogenous state for either the B<sup>0</sup> or B<sup>+</sup> thalassemia gene or, less commonly, the compound heterozygous state for the two genes.

### Clinical Features

The newborn infant with Beta thalassemia is not anemic.(within first year of life and in severe cases within a few weeks of birth. Anemia (insidious) but in severe anemia cardiac dilatation is present. Splenomegaly and hepatomegaly are common.

Changes of skeletal system (Mongoloid facies) are due to expansion of the marrow in the malar bones, and X-ray change in skull, long bones, hands and feet.

Severe infection (pericarditis), hypersplenism, anemia, and thrombocytopenia are also common.

Organ dysfunction due to increase in body iron secondary to frequent transfusions and increase intestinal absorption lead to pancreatic hemosiderosis (DM, Cirrhosis) or/and cardiac hemosiderosis (CCF, arrhythmias, Heart block).

### Blood Picture

Blood picture resembles iron deficiency anemia. Hb of 3-9 g/dl.

Blood smear characterized by presence of anisocytosis and poikilocytosis, which are often bizarre. Microcytosis (predominant), polychromasia and punctate basophilia are usually present. Hypochromia is a striking feature. Target cells are prominent. Tear drop cells often seen. Some cells are macrocytic. Occasionally spherocytic RBCs are present. Normoblast present in large number in postsplenectomy patients.

MCV and MCH are significantly reduced. MCHC also reduced. Reticulocyte increased in 10% or more. WBC is increased of 15-40,000/ $\mu$ L or more with shift to left. Some myelocytes are commonly present. Platelets count is normal, but may be reduced in hypersplenism.

The osmotic fragility decreased. (Increase resistance to hemolysis). There is evidence of intravascular hemolysis. The serum bilirubin slightly increased. Haptoglobin decreased.

Dark-brown urine is commonly observed. Serum uric acid frequently elevated and clinical gout may occur.

The serum iron and ferritin are invariably elevated and transferrin completely saturated.

Serum and red cells folate is often reduced.

### Bone Marrow Aspiration

Hyperplastic erythropoiesis is proportional to the anemia. Increase proportion of basophilic and polychromatic normoblast (micronormoblast). Siderotic granules are commonly scattered through the cytoplasm of the normoblast. Ring sideroblast occasionally seen.

### Hb-Electrophoresis

HbF (10-98) estimated also by acid elution and alkali denaturation tests.

HbA very little or absent and HbA<sub>2</sub> is variable



## Treatment of Thalassemia

**1. Packed RBCs transfusion:** 10- 15 ml/kg every 4-6 weeks to keep Hb level above 10 gm/dl (Hypertransfusion). Recently, a more extensive program of transfusion aims to keep Hb above 12 gm/dl (supertransfusion) The packed RBCs should be better washed with saline and carefully cross-matched.

Recently, neocyte transfusions (Juvenile RBCs separated by special techniques), and single donor transfusions have been shown to be more advantageous to the patient. These RBCs survive longer.

**2. Iron chelation therapy:** Is essential especially in the high transfusion programs, to prevent "Hemosiderosis".

(a) Desferal: is given IV, IM or SC injection. Most recently IgM is given by continuous SC infusion, using an electric infusion pump for 12h/d, repeated 4 times/week. This permits a negative iron balance.

(b) **SHAM:** SHAM is a new promising oral iron-chelating agent of salicyl Hydroxamic acid). It is given orally 20-40 mg/kg/day in divided doses, 5 days every week, which minimizes the discomfort of injections as in desferal. It may lead to hypocalcemia, as it also chelates calcium. Therefore, Calcium intake should be increased in the other 2 days of the week. It is less active in iron chelation than desferal.

Both drugs cause red discoloration of the urine (passage of iron chelates).

Exjade approved in 2005, orally ingested. highly bioavailable chelator that is absorbed in the GI tract. Dose-dependent  $t_{1/2}$  of 12-18 hours given once daily 20-40 mg/kg

2. Fetal hemoglobin stimulator are : 5-Azacytidine/decitabine ,butyrate, pomalidomide(animal models) and combinations of different compounds have also been tried with some success

**4. Splenectomy:** Is indicated only if there are manifestations of hypersplenism. This presents by clinical and laboratory manifestations of pancytopenia. There is an increased need for frequent transfusions. A patient who does need more than 300 ml packed RBCs/kg/year is said to have hypersplenism. It should be postponed after 5 years of age

5. Folic acid: 1mg/day prevent megaloblastic crises.

6. Never give iron to these patients. They already have iron over-load.

7. Thalassemia minor requires no treatment.

8. HSC Transplantation is the only cure for thalassemia patients. It should be considered in all patients who have an acceptable donor. Patients are classified on the basis of their risk factors which include: inadequate chelation, presence of liver fibrosis and hepatomegaly

9. Gene therapy: Why  $\beta$ -thalassemia, SCD are excellent candidates for genetic approaches: Monogenic disorders and it could be cured by introducing or correcting a single gene

## REVIEW QUESTIONS

1. Bart's hydrops fetalis is lethal because
  - a. Hb Barts cannot bind oxygen
  - b. The excess  $\alpha$ -globin chains form insoluble precipitates
  - c. Hb Barts cannot release oxygen to tissues
  - d. Microcytic red cells become trapped in the placenta
  
2. HbS that are composed of four  $\gamma$ -chain ( $\gamma_4$ ) and four  $\beta$ -chain ( $\beta_4$ ) are respectively.
  - a. Hbs H and C
  - b. Hbs H and Barts
  - c. Hbs Barts and H
  - d. Hbs Barts and S
  
3. Homozygous  $\beta$ -thalassemia can be confused with iron deficiency because both have.
  - a. Decreased serum ferritin
  - b. Decreased serum iron
  - c. Decreased % transferrin saturation
  - d. Microcytic, Hypochromic RBC
  
4. Hb(s) that migrate with HbS on cellulose acetate PH (is) are
  - a. Hb constant spring
  - b. Hb Barts
  - c. Hb H
  - d. Hb Lepore
  
5. The abnormally increased Hb electrophoresis value(s) that would usually exclude the possibility of  $\alpha$ -thalassemia is (are)
  - a. HbA<sub>2</sub> and HbF
  - b. HbA<sub>2</sub> and HbH
  - c. HbF and HbH
  - d. Hb-Barts
  
6. HbH inclusion bodies may
  - a. Found in  $\beta$ -thalassemia
  - b. Be seen on Wright-stained blood film
  - c. Appear eccentrically near the red cell membrane
  - d. Make red cell look like "pitted golf balls"
  
7. What is the clinical manifestation of  $\alpha$ -thalassemia with sickle cell anemia?
  - a. Severe, life threatening anemia
  - b. Relatively asymptomatic until placed in oxygen deprived environment.
  - c. Less severe than sickle cell anemia alone
  - d. Skeletal abnormality, but milder anemia than sickle cell anemia

## HEREDITARY HEMOLYTIC ANEMIA

# 7

### HEREDITARY SPHEROCYTOSIS

HS is a relatively common hemolytic anemia due to an intrinsic defect of the red cell membrane and abnormalities of spectrin protein of the membrane, which results in the cell being of spherocytic shape.

Spherocyte have a decreased surface area-to volume ratio and are more rigid and less deformable than normal cells.

The membrane abnormality is associated functionally with an increased permeability to sodium. An increased rate of passive movement of sodium into the cell is compensated for by an increased rate of active transport of sodium out of the cell by the cation pump mechanism, which requires ATP derived from red cell glycolysis.

#### Clinical Feature

It is inherited as an autosomal dominant trait. Male and female are equally affected.

Anemia or jaundice or both are common. Gallstone/or splenomegaly (less frequent).

Jaundice moderate with increase of serum bilirubin. There is no bile in urine but may contain urobilinogen.

- **Hemolytic crises**
  - Increase depth of jaundice
  - Darkening of urine
  - Increase size of spleen
- **Aplastic crises**
  - Bilirubin decrease
  - Reticulocyte count decreased (zero)
  - Folate deficiency

#### Blood Picture

Anemia with spherocytosis (Hb 7-12 g/dl), increase reticulocyte count, serum bilirubin level and erythrocyte osmotic fragility. The antiglobulin test is negative.

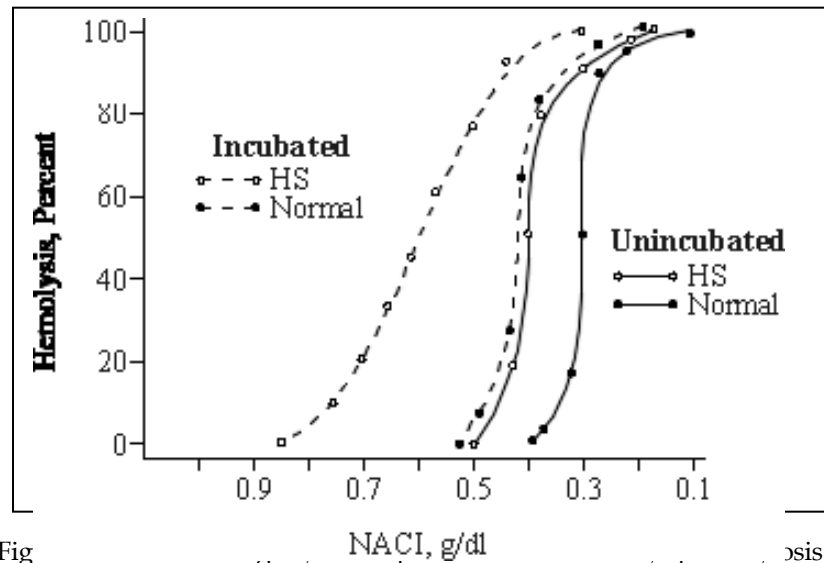
**Blood Film** shows spherocytosis are usually numerous and contrast sharply with the polychromatic macrocytes. A small number of normoblasts may be present in high reticulocyte count. MCV is usually normal but is occasionally slightly reduced.

MCH is normal but the MCHC is often increased ranging from 34-40%. Reticulocytes increased 5-20%.

The normal erythrocytes's pale center is absent. Because of their spheroidal shape, which is due in part to loss of membrane area, they are more susceptible to osmotic stress as measured by the osmotic fragility test (Figure 7.1). To perform this test, red cells are suspended in saline solutions of various tonicities. At lower tonicities water enters and swells the red cells, ultimately causing them to lyse. This normally occurs at a sodium chloride concentration of 0.55 percent. In patient with HS and in patients with other diseases in which large numbers of spherocytes are produced.



Lyse may begin at 0.70% sodium chloride concentration or even higher. This increased susceptibility to osmotic lysis is accentuated by prior incubation of the red cells for 24 hours at 37°C. Both normal subjects and HS patients will have increased osmotic fragility after incubation, but the effect is more marked for the patients with hereditary spherocytosis, whose cells may begin to lyse in 0.80% sodium chloride.



Fig

Autohemolysis is the amount of spontaneous lysis, which occurs on sterile incubation for 48 hours at 37°C. It is increased upto 5-7% (normally <4%). The increased autohemolysis is substantially reduced, but usually not completely corrected, by the addition of glucose.

The survival in the patient's circulation of autologous red cells labelled with  $^{51}\text{Cr}$  is shortened and surface counting of radioactivity reveals excessive uptake over the spleen.

#### Treatment

1. Splenectomy to correct the hemolytic process and prevent crises, but it does not correct the underlying defects. It should be followed by severe infection complications. The most severe is "overwhelming sepsis" due to pneumococci and Hemophilus influenza, in addition to salmonella osteomyelitis. These serious infections occur frequently with early splenectomy, or in case of sickle cell anemia due to early autosplenectomy. Therefore splenectomy should be postponed till the patient is over 5 year's age. Vaccination by polyvalent pneumococcal vaccine, and hemophilus influenza group B is better given before splenectomy.
2. Prophylactic long acting penicillin therapy after splenectomy is advised.
3. Packed RBCs transfusions for correction of anemia and aplastic crises till splenectomy is done.
4. Give folic acid prophylactically for severe cases
5. If cholecystectomy required, perform splenectomy also to reduce risk of recurrent gallstone

## HEREDITARY ELLIPTOCYTOSIS

- Autosomal dominant
- Abnormality in spectrin interaction with other membrane proteins
- 25-75% elliptocytes
- Hemolysis is usually mild
- Treatment, splenectomy for severe hemolysis. Immunize against pneumococcal first.

## GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY

It is an X-linked recessive disease, affecting males and much less commonly females.

It results from deficiency of glucose-6-phosphatase dehydrogenase enzyme, which catalyzes the first reaction in the Hexose monophosphate (HMP) pathway. This pathway is essential for production of reduced NADPH<sub>2</sub>, which is necessary for conversion of oxidized to reduced glutathione. This protects the RBCs against oxidizing agents.

If the enzyme is deficient, exposure of RBCs to oxidizing agents lead to hemolysis as:

- (1) Antipyretics (Salicylate, Aspirin) (2) Antimalarial drugs (3) Sulfonamides
- (4) PAS (5) Synthetic Vitamin K (6) Naphthalene (7) chloramphenicol, nitrofurantoin
- (8) Fava bean and its derivatives

### Clinical Picture

Acute hemolysis occurs about 48-96 hours after ingestion of an oxidizing agent or infection. It manifests by abdominal pain, nausea, vomiting and hemoglobinuria.

Anemia and jaundice occur in severe cases. Death may occur in severe cases.

Neonatal hemolysis, jaundice and kernicterus may occur in affected males.

The disease is due to inheritance of an abnormal G-6-PD gene on X-chromosome, which leads to production of a fragile G6PD enzyme, or an enzyme with weak activity. More than 200 variants of the enzyme could be recognized in different localities of the world, with variable degrees of enzymatic activity.

### The Most Common Clinical Forms

1. G-6-PD A: common among American blacks: enzymatic activity is 5-15% of normal, causes a less severe self limited hemolysis.
2. G-6-PD B: common in mediterranean area; enzymatic activity is less than 5% of normal, and leads to severe hemolytic attacks.
3. G-6-PD canton: common in Chinese

### **Laboratory Finding**

Blood smear shows red cell with absent hemoglobin (bite and blister cells), polychromasia, basophilic stippling, spherocytosis. Heinz bodies may be seen in a reticulocyte preparation with supravital staining. Heinz body test also give positive result.

Screening test for red cell G6PD deficiency measures the generation of NADPH. The enzyme may also be characterized by electrophoresis, assay of activity and DNA analysis.

### **Treatment**

#### **A. Prophylactic:**

It is advisable to avoid of the precipitating agents in susceptible persons.

In febrile patients; give paracetamol (avoid salicylate). Vitamin E in pharmacologic doses (400-800 u/d) protects against hemolysis, due to its antioxidant action.

#### **B. Curatives:**

- Any offending agent should be stopped
- Underlying infections should be treated
- In mild cases: Observation for progress of anemia
- If severe: Emergency packed RBCs transfusion of 10-20 ml/kg. Affected individuals are treated with oxygen and bed rest, which may afford symptomatic relief.

### **PYRUVATE KINASE DEFICIENCY**

Pyruvate kinase (PK) deficiency is the most frequent enzyme deficiency in the Embden-Meyerhof (glycolytic) pathway to cause chronic non-spherocytic hemolytic anemia (CNSHA). Inheritance is autosomal recessive. The O<sub>2</sub>-dissociation curve is shifted to the right so symptoms are mild in comparison to the degree of anemia. Splenectomy partly improves the anemia.



## REVIEW QUESTIONS

1. Which of the following statements about hereditary spherocytosis is incorrect?

- a. The disease is most commonly associated with autosomal dominant inheritance
- b. The RBCs characteristically have increased osmotic fragility.
- c. After splenectomy the RBCs remain spherocytic
- d. Splenectomy seldom relieves the anemia

2. Which of the following would not be expected to be associated with hereditary spherocytosis?

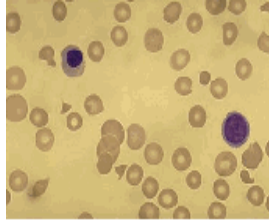
- a. Cholelithiasis
- b. Cyanosis
- c. Splenomegaly
- d. Jaundice

3. A possible source of error in quantitative enzyme assays for G6PD or PK is

- a. Hemolysis contamination with leukocytes that contain higher G6PD and PK enzyme activity than red cells.
- b. Recent transfusion which may obscure a G6PD or PK enzyme deficiency
- c. Hemolysate contamination with platelets that have high G6PD and PK enzyme activity.
- d. All of the above.

## ACQUIRED HEMOLYTIC ANEMIA

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## PAROXYSMAL NOCTURNAL HEMGLOBINURIA

PNH is a rare disorder characterised by attacks of acute intravascular hemolysis during sleep (some times not associated with sleep). A red cell defect renders one population of red cells markedly sensitive to complement. The degree of hemolysis varies as a consequence of population of abnormal stem cells.

PNH begins insidiously in-patients between the age of 30 and 60 years. Irregular episodes of hemoglobinuria associated with sleep are a starting manifestation of this disorder.

### Laboratory Finding

#### Blood Picture

Anemia, macrocytosis, polychromasia  
Reticulocytosis, moderate leukopenia, mild thrombocytopenia  
HbF occasionally raised  
Hyperbilirubinemia  
Neutrophil alkaline phosphatase is low  
Hypercoagulability  
Direct coomb's test

### Further Investigations

Hemoglobin, hemosiderinuria

Serological test

A) Ham's acid serum test: Patients red cell will undergo lysis in compatible acidified serum at 37c (serum may be patient own) 10-15% lysis implies a positive test with patient own serum.

B) Sucrose hemolysis test: Isotonic solution of low ionic strength if causes hemolysis of more than 10% cells, indicates of PNH.

## Treatment

Patients have an average life expectancy of more than 10 years. Treatment includes blood transfusion therapy, antibiotics and anticoagulants. If an induced marrow aplasia exists and the patient is younger than 50 years of age, bone marrow transplantation may be a consideration.

Eculizumab is the only Food and Drug Administration–approved drug for the treatment of PNH. Thus, when to initiate therapy rather than what therapy to initiate is often the most important clinical question. Given that eculizumab is expensive, does not eradicate the PNH clone, and must be given lifelong, it is best reserved for the symptomatic patient with a large percentage of PNH cells or the PNH patient with thrombosis irrespective of the PNH clone size

## RED CELL FRAGMENTATION SYNDROMES

### Classification

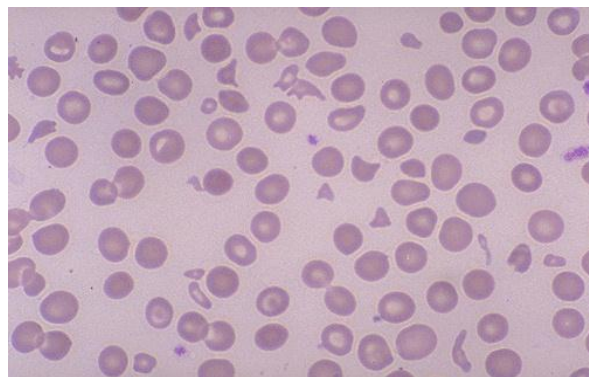
1. Cardiac and large vessel abnormalities
2. Small vessel disease (microangiopathic)
  - a. Thrombotic thrombocytopenic purpura (TTP) hemolytic uremic syndrome (HUS)
  - b. DIC
  - c. Metastatic carcinoma
  - d. Preeclampsia sepsis
  - e. Malignant hypertension
  - f. Vasculitis
3. Infection (Malaria, Clostridium)
4. Thermal injuries

### Diagnosis

Evidence of hemolysis, (schistocytes, polychromasia, thrombocytopenia), hemosiderinuria and hemoglobinuria

### Treatment

Treatment of underlying disease, replace iron if indicated.



**Figure 8.1 :** Numerous RBCs appears as “helmet” cells such as fragment RBCs “schistocytes”. Blood smear of patient with microangiopathic hemolytic anemia (MAHA).



## **MARCH HEMOGLOBINURIA**

This is due to damage to red cells between the small bones of the feet, usually during prolonged marching of running. The blood film does not show fragments.

## **AUTOIMMUNE HEMOLYTIC ANEMIA**

AIHA is identified by the presence of (Auto)-antibodies that react with RBCs, this Abs are detected on the RBCs by the coomb's test.

### **COOMB'S TEST**

#### **Principle**

Certain antibodies combine with red cells but are unable to bring about agglutination because they are too small to link together adjacent cells, which are repelled from one another by their negative surface charges.

There are 2 kinds of antiglobulin test:

#### **1. Direct Coomb's Test**

Rabbits are immunized with whole human serum containing gamma globulin. This yields rabbit antiserum. When red cells coated with incomplete antibodies are suspended in the appropriate coomb's antiserum, agglutination of red cells results.

Direct coomb's test is useful in the diagnosis of hemolytic diseases of the newborn and autoimmune hemolytic anemia and in the investigation of transfusion reactions, very high reticulocyte count, lead poisoning, drug induced hemolysis and some viral diseases have at times caused positive direct antiglobulin reaction.

#### **2. Indirect Coomb's Test**

It is employed to detect free antibodies not fixed to red cells. In this test normal red cells are washed repeatedly with saline to remove non-specifically adherent gamma globulin. Next, red cells are treated with a proteolytic enzyme such as papain, which modifies the red cells envelope and renders them more avid to bind incomplete antibodies. Lastly, the sensitised red cells are incubated in patients serum and rabbit antiserum added. In presence of free antibody agglutination occur.

The indirect antiglobulin test is that used in detecting incompatibility during cross matching test, in detecting and identifying irregular antibodies not identifiable by other means.

### **Warm-Ab Hemolytic Anemia**

It is the most common form of AIHA due to presence of warm antibodies that cause hemolysis of red cells at body temperature and coomb's test is positive.

Antibodies are usually of the IgG type.

## **Causes**

### **A. Idiopathic**

### **B. Secondary**

## **Secondary causes**

1. Lymphoma, CLL, Hodgkin's disease and sarcoidosis
2. Carcinoma, ulcerative colitis, ovarian cyst.
3. SLE and polyarteritis nodosa.
4. Drugs: Methyldopa, penicillin, stibophen, quinine and quinidine.

## **Clinical Features**

1. Features of the underlying causes.
2. Hemolytic anemia
3. Splenomegaly is nearly always present
4. Thrombophlebitis is a frequent complication.

## **Investigations**

1. RBC: Normocytic normochromic anemia
2. Features of hemolysis, fragility is commonly increased
3. Spherocytosis is common
4. Coomb's Test is Positive

## **Treatment**

1. Treatment of the underlying cause
2. Corticosteroid as prednisone is usually effective
3. Immunosuppressive drugs- in resistance cases e.g cyclophosphamide, 6-mercaptopurine and azathioprine
4. Splenectomy maybe helpful after failure of corticosteroid therapy and when there is evidence of hypersplenism.
5. Blood transfusion when Hb falls 25%

## **COLD AB HEMOLYSIS AND PAROXYSMAL COLD HEMOGLOBINURIA**

Autohemolytic attack may follow local or general exposure to cold. Pain in the back, legs or abdomen, cramps and other symptoms of acute hemolysis such as chills, fever and malaise are associated with the passage of dark brown urine together with jaundice. The direct coomb's test is positive only during the attacks, and becomes negative thereafter. Antibodies are usually of IgM type. The frequent conditions are syphilis, viral infection (primary atypical pneumonia), infectious mononucleosis, cytomegalovirus, lymphoma and macroglobulinemia.

## **Diagnosis**

- Positive cold agglutination test best at 4°C
- Positive direct coomb's test for complement at any temperature
- Agglutination in the blood film

### **Treatment**

- Treatment of the underlying causes
- Avoid exposure to cold
- Immunosuppressive agents
- Plasmapheresis

## **DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA**

1. Drug may cause immune hemolytic anemias by three different mechanisms:

Antibody directed against a drug- red cell membrane complex (e.g penicillin or cephalothin)

2. Deposition of complement via drug-protein (antigen)-antibody complex onto red cell surface (e.g quinidine or chlorpromid).

3. An autoimmune hemolytic anemia in which the role of the drug is mysterious e.g methyldopa.

## **HEMOLYTIC DISEASE OF THE NEWBORN**

It is due to a reaction between the Rh factor, an antigen on the surface of red cells of the fetus (antigen D) and corresponding agglutinating antibodies, which reach the fetal blood from the maternal circulation (anti-D).

The fetal antigen cannot cross the intact placental barrier and does so only shortly before and during delivery due to transplacental hemorrhage, where it stimulates the formation of antibody against it in the mother. Accordingly, the first child usually escapes hemolysis while the formed maternal antibodies affect the following children if they are Rh+ (homozygous (DD) or heterozygous (Dd). If the mother has been previously transfused by an Rh+ blood even in childhood, a dangerously high response may occur during a first pregnancy.

### **Clinical Features**

Three grades of severity are recognized:

1. Erythroblastosis fetalis: mild form
- Anemia, jaundice (maybe), hepatosplenomegaly
2. Icterus gravis neonatorum:
- Jaundice (80% of babies) within a week of birth, liver cell failure
  - Neurological lesions
3. Hydrops fetalis: Severe form, edema, ascites and sign of hypoxia

### **Treatment**

1. Exchange transfusion of blood. Keep bilirubin below 18 mg/dl

A polythene catheter is passed along the umbilical vein into the inferior vena cava and small quantities of the infants' blood are successively



withdrawn and replaced by an equal volume of compatible Rh-negative blood. This procedure can be repeated if serum bilirubin exceeds 18 mg/dl.

2. Prevention; To bring about destruction of Rh<sup>+</sup> fetal red cells in maternal blood, anti-D serum in the form of gamma globulin containing a high titre of anti-d is given to the mother soon after delivery within 24 hours.

## REVIEW QUESTIONS

1. Which of the following would be LEAST likely to be associated with warm antibody-mediated hemolysis?
  - a. Chronic lymphocytic leukemia
  - b. Ulcerative colitis
  - c. SLE
  - d. Osteodystrophy
2. Which of the following infections have been associated with paroxysmal cold hemoglobinuria?
  - a. Infectious mononucleosis
  - b. Syphilis
  - c. Cytomegalovirus
  - d. All of the above
3. Which of the following is not typical of paroxysmal nocturnal hemoglobinuria (PNH)?
  - a. Hemolytic anemia
  - b. Leukemia
  - c. Reticulocytosis
  - d. Thrombocytosis
4. Which list is the most complete for clinical features of PNH?
  - a. Hemoglobinuria, hemosiderinuria, abnormal pain, headache and backache
  - b. Weakness, fatigue, hepatosplenomegaly, backpain
  - c. Recurrent infection, skin ulcers, spontaneous fractures, dental problems.
  - d. Persistent bacterial infections, jaundice, kernicterus and weight loss.
5. Which of the following is not usually a treatment for PNH?
  - a. Anticoagulant therapy
  - b. Blood transfusion/marrow transplant
  - c. Adrenocorticosteroids
  - d. Immunosuppressive therapy
6. What is deficiency causes hemoglobin to be oxidized from the ferrous to the ferric state?
  - a. G6PD deficiency
  - b. PK deficiency
  - c. NADH-methemoglobin reductase deficiency
  - d. Lactate dehydrogenase deficiency

7. What is the most common glycolytic deficiency associated with pentose phosphate pathway (aerobic pathway)?
- Pyruvate kinase
  - Glucose 6 phosphate dehydrogenase
  - Glutathione reductase deficiency
  - Hexokinase deficiency
8. In the evaluation of a patient for G6PD deficiency, which of the following test results would indicate a deficiency of the enzyme?
- Increased formation of Heinz bodies
  - Lack of fluorescence in the fluorescenet spot test
  - Failure to reduce methemoglobin in the presence of methylene blue
  - All of the above

Questions: Match the following

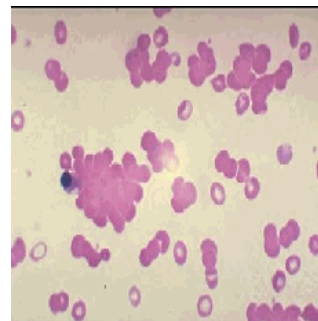
- The test is highly specific for PNH, but somewhat insensitive
- A positive test may be found with autoimmune hemolytic anemia disease
- A positive test is associated with G6PD deficiency.
- Generally used as the screening test for PNH because of its simplicity.

- Sucrose lysis test (sugar water test)
- Acidfied serum test (Ham test)
- Both
- Neither

### Q 13: Q UIZ

A 67 year old woman spent two hours shoveling snow from her driveway after an unexpected blizzard left her stranded in her home. The next morning, she was shocked to see that her urine was the color of beet juice. Investigations: Blood smear  
The correct answer:

- Paroxysmal cold hemoglobinuria
- Cryoglobulinemia
- Cold agglutinin disease
- Paroxysmal Nocturnal hemoglobinuria



The macrocytic anemias can be divided into two categories (1) Those that are not megaloblastic and (2) Those that demonstrate megaloblastic changes in both the bone marrow and the peripheral blood. The non-megaloblastic group includes anemias in which the red cell size is increased but there is a normoblastic morphological appearance. Examples include anemias secondary to alcoholism and certain hemolytic processes.

### MEGALOBLASTIC ANEMIA

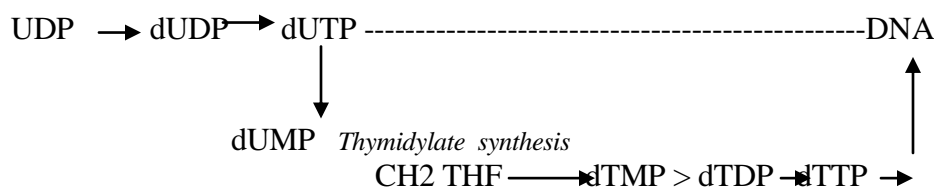
The pathophysiology of the megaloblastic anemias is associated with two primary abnormalities: (1) ineffective erythropoiesis and (2) a moderate hemolysis of circulating erythrocytes.

The etiological classification can be divided into three main groups (1) those due to vitamin B12 deficiency (2) those due to folic acid deficiency; and (3) those that are unresponsive treatment with either of these essential nutrients and result from a variety of causes.

#### Biochemistry

The defective nuclear maturation and the megaloblastic morphology are caused by a decrease in thymidine triphosphate (TTP) synthesis from uridine monophosphate (UMP). This deficiency interferes with nuclear maturation, DNA replication, and cell division. When thymidine triphosphate is not present in adequate amounts, deoxyuridine triphosphate incorporates into the DNA instead. This misincorporation causes fragmentation of the nucleus and ultimately immature cell destruction.

The primary causes for lack of thymidine and consequently defective DNA synthesis are vitamin B12 and folic acid deficiencies. These vitamins, in the form of cofactors, play important roles in some key reactions involved in DNA synthesis. In addition, drugs that interfere with the metabolism of these vitamins also cause DNA impairment.



**Figure 9.1:** Thymidine synthesis pathway from uridine nucleotide. Uracil is incorporated into DNA in the absence of thymidine. UDP= uridine diphosphate, dUDP= deoxyuridine diphosphate; dUTP= deoxyuridine triphosphate; dUMP= deoxyuridine monophosphate; dTMP= deoxythymidine monophosphate; dTDP= deoxythymidine diphosphate; dTTP= deoxythymidine triphosphate; CH2 THF= methylene tetrahydrofolate.



## **Classification**

### **1. Vitamin B12 Deficiency**

### **2. Folate Deficiency**

### **3. Megaloblastic anemia unresponsive to vitamin B12 and Folate therapy**

### **1. Vitamin B12 Deficiency**

1. Decreased in intake
  - A. Dietary, usually seen only in true vegetarians
  - B. Impaired absorption, such as in pernicious anemia
  - C. Malabsorption (Familial, drug induced, sprue, celiac disease, gastrectomy)
  - D. Competition from parasites (Fish tapeworm and bacteria)
2. Increased requirements
  - A. Pregnancy
  - B. Increased cellular proliferation (tumors)
  - C. Hyperthyroidism
3. Impaired utilization
  - A. Red cell enzymopathy
  - B. Abnormal vitamin B12 binding protein (transcobalamin II)
  - C. Nitrous oxide administration
  - D. Lack of transport protein (TcII)

### **2. Folate Deficiency**

1. Decreased in intake
  - A. Dietary, usually a lack of green vegetables
  - B. Alcoholism
  - C. Impaired absorption due to sprue and celiac disease
2. Increased requirements
  - A. Pregnancy
  - B. Increased cellular proliferation (tumors)
  - C. Miscellaneous states (Homocystinuria, hyperthyroidism)
3. Impaired utilization
  - A. Folic acid antagonists ( methotrexate, dilantin, trimethoprim, pyrimethamine)

### **Megaloblastic anemia unresponsive to vitamin B12 and Folate therapy**

#### **1. Metabolic inhibitors**

- A. Drug-induced disorders: Purine antagonists (6 mercaptopurine), pyrimidine antagonists (5-Fluorouracil) alkylating agents (cyclophosphamide).
2. Unknown causes
  - A. Pyridoxine-responsive megaloblastic anemia
  - B. Erythemic myelosis (Erythroleukemia or DiGuglielmo syndrome).

**Table 9.1: Vitamin B12 and Folate metabolism**

VITAMIN B 12		FOLATE
Content in food	vegetables: Poor	Vegetables:rich
Meat :	Rich	Meat : Moderate
Effect of cooking	10-30% loss	60-90% loss
Adult daily Require	2-4 µg	200 µg
Adult daily intake	5-30 µg	100-500 µg
Site of absorption		ileum
Duodenum/jegnum		
Body stores	2-5 mg	5-20 mg

**VITAMIN B12 DEFICIENCY**

The human body contains between 2000 and 5000 µg vitamin B12 and has a daily requirement of about 2 µg. Thus, the body requirement of a person who develops a defect of vitamin B12 absorption can be supplied for a considerable period of time from the tissue stores. Clinical manifestations of deficiency develop only when the tissue stores are almost completely exhausted.

Vitamin B12 in food is bound in the acidic environment of the stomach to be released. In the stomach, the food free initially binds to salivary haptocorrin (a vitamin B12 binding protein formerly known as R-binder), only to be re-released in the duodenum after pancreatic enzymes degrade the haptocorrin.

In the duodenum, the free vitamin B12 combines with another B12-binder (Intrinsic factor) secreted by the parietal cells of the stomach. Vitamin B12 is absorbed at the terminal ileum, and then only when it is bound to intrinsic factor. Idiopathic deficiency of intrinsic factor is called pernicious anemia. Once absorbed, vitamin B12 is freed from the B12-intrinsic factor complex and released into the blood, where it is transported by a specific carrier protein, transcobalamin II. However, 80% of plasma vitamin B12 is bound to other serum haptocorrins (transcobalamin III and I). The transcobalamin II-B12 complex is carried to cells and is pinocytosed via transcobalamin II receptors. Intracellularly, vitamin B12 joins forces with folate and assists in DNA synthesis.

**Clinical Manifestation**

There are three cardinal manifestations of vitamin B12 deficiency of whatever cause:

- Macrocytic megaloblastic anemia
- Glossitis
- Peripheral neuropathy and subacute combined degeneration of the spinal cord. Optic neuropathy, depression, and impaired memory.

On examination: Sensory loss in the legs and positive Romberg sign.

Neurological abnormalities appear to occur more frequently in pernicious anemia due to vitamin B12 deficiency.

### Special Tests in Diagnosis

The main test for the detection of vitamin B12 deficiency is the serum vitamin B12 assay. To establish the cause of the deficiency, a radioactive vitamin B12 absorption test is performed.

Serum vitamin B12 assay:

1. Microbiological assay
2. Radio-isotop assay

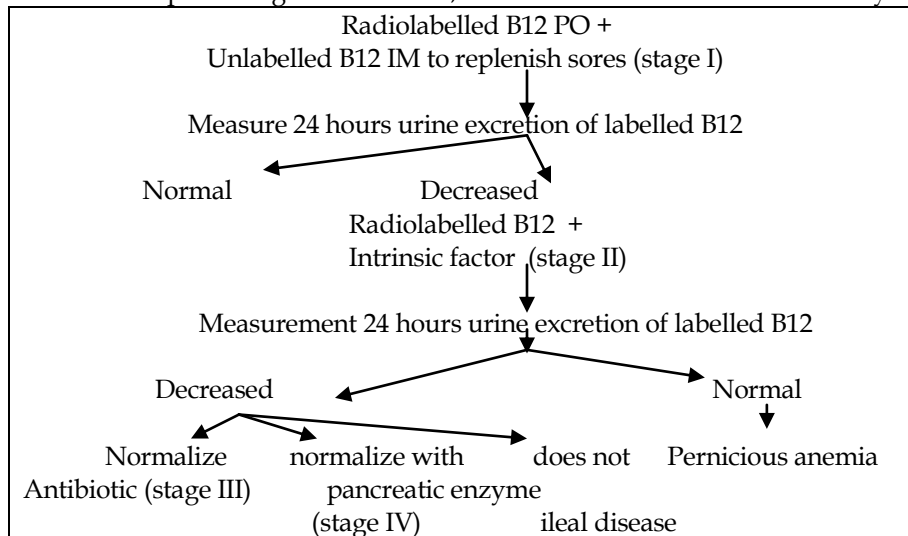
Radioactive vitamin B12 absorption test (eg Schilling Test):

The ability of the body to absorb vitamin B12 can be assessed by measuring the absorption of a small oral dose of  $^{57}\text{C}$ -labelled vitamin B12 orally if simultaneous administration of intrinsic factor, it implies lack of intrinsic factor.

### The Schilling Test

An oral dose of  $1\mu\text{g}$  radioactive vitamin B12 ( $^{57}\text{C}$ -vit.B12) is administered to the fasting subject followed two hours later by a large parenteral injection of unlabelled B12 ( $1000\mu\text{g}$ ). The injection flushes out about 1/3 of the absorbed radioactive B12 into the next 24 hours.

- Normal subjects excrete 10% or more of the  $1\mu\text{g}$  dose in their urine.
- Patients with pernicious anemia excrete less than 5% but occasionally upto 7% of the dose.
- Borderline results of upto 10% may occur in atrophic gastritis.
- If the patients absorb normal amounts of vitamin B12, no further testing is necessary.
- If absorption is subnormal, a second parenteral injection of unlabelled B12 is given 24 hours later, followed by a further test dose of radioactive B12 with intrinsic factor, and the B12 absorption is again estimated. If absorption returns to normal, a diagnosis of pernicious anemia may be made.
- If absorption is again subnormal, a lesion of the small intestine is likely.



Measurement of the unsaturated B12 binding capacity (UBBC), which in the normal subject reflects the amount of TC II and to a lesser extent TC I and TC II and TC III available in the serum for binding with added B12. The normal range for serum UBBC is 500-1200 ng/l. The UBBC is usually elevated due to an increase in TC I in CML, acute promyelocytic leukemia.



## FOLATE DEFICIENCY

Folate is abundant in yeast, many leafy vegetables and organ meats such as liver and kidneys. An ample amount of folate is present in most well balanced diet. The human body stores little folic acid. Storage amounts of a complete absence of dietary folates existed. However, a chronically inadequate diet can produce folic acid deficiency. However, folic acid antagonist, such as certain drugs used to treat leukemias, and oral contraceptives appear to reduce the absorption of folic acid.

Dietary folic acid is in the polyglutamate form, which is deconjugated to the monoglutamate form during absorption. In the mucosa, folic acid undergoes complete reduction by dihydrofolate reductase into tetrahydrofolate. It is then methylated and released into the blood and transported (No specific carrier protein) into the target cells where it transfers its methyl group to homocysteine to form methionine and tetrahydrofolate. This reaction is made possible by the enzyme methionine synthetase, which requires vitamin B12 as a cofactor. Tetrahydrofolate is used in the transfer of one-carbon fragment from donors such as a serine to DNA bases.

### Causes of Folate Deficiency

1. Inadequate intake
2. Intestinal malabsorption (e.g celiac disease, tropical sprue)
3. Increased demand: Pregnancy, hemolytic anemia, leukemia, lymphoma, sideroblastic anemia, carcinoma, inflammatory disorder, hyperthyroidism and skin disease.
4. Inability to utilize folate due to the action of folate antagonist (anticonvulsant, contraceptives).

### Manifestations

The most typical manifestation of folate deficiency is megaloblastic anemia, a hematopoietic disorder whose features have already been discussed. The salient molecular abnormality in folate deficiency is a marked slowing of DNA synthesis, a defect expressed not only in the characteristic morphological abnormalities of the megaloblastic cells, but also in a marked prolongation of the S (DNA synthesis) phase in replicating cells and in a disruption in chromatin structure detectable as chromosomal tangles and breaks. Despite extensive study, a biochemical explanation for the slowing of DNA synthesis in folate deficiency is not yet available, although it appears to be somehow related to the defect in dTMP formation seen in folate-deficient cells.

**OTHER MANIFESTATIONS:** Folate deficiency affects other rapidly dividing tissues. A stomatitis characterized by a sore mouth and a smooth, beefy red tongue occurs frequently. It probably results from impaired proliferation of the oral mucosa, which is worn away during eating and must be constantly renewed.

**Special Tests in Diagnosis:** There are two main laboratory tests used to detect folate deficiency

**Serum Folate Assay:** Microbiological (*Lactobacillus*) and radioisotope method are available for measuring serum folate concentration.

### **Red Cell Folate Assay**

Red cell contains 20-50 times as much folate as serum. The red cell folate level is usually a more reliable indicator of tissue folate stores than the serum folate, which fluctuates widely according to dietary intake. Microbiological or radioisotope assay method may be used.

Low red cell folate levels are found in patients with megaloblastic anemia due to folate deficiency.

Figlu test (Determination of formiminoglutamic acid in urine) is an intermediary metabolic product of histidine metabolism and is normally metabolized to glutamic acid, with the help of tetrahydrofolic acid (THFA). In folic acid deficiency THFA is not available and FIGLU is found in the urine in increased amounts. The sensitivity of the test can be increased by the histidine-loading technique. This test however is not very specific.

Radioactive folic acid test is diagnostic (like schilling test in B12 deficiency).

### **Peripheral Blood Picture in Megaloblastic Anemia**

1. Anemia with marked oval macrocytosis and elevated MCV. The higher is the MCV, the greater the incidence of megaloblastosis. MCV values above 125 fl are almost always associated with vitamin B12 or folate deficiency and a frankly megaloblastic bone marrow.

2. Neutropenia with hypersegmented neutrophils.

3. Mild, usually symptomless, thrombocytopenia.

The earliest change is the development of macrocytosis and an elevated MCV without anemia. Anemia then develops weeks, months or rarely years later and as the level of hemoglobin falls. Anisocytosis, macrocytosis and poikilocytosis become more prominent. Finally neutropenia and thrombocytopenia develop.

Neutrophil hypersegmented is present when more than 5% of neutrophils have 5 lobes or the film shows at least one six-lobed cell. Hypersegmentation is an early sign of vitamin B12 or folate deficiency, and is useful in the diagnosis of megaloblastosis with minimal or no anemia (other conditions with hypersegmentation are I.D.A, myeloproliferative syndrome and chronic renal failure).

### **Bone Marrow Morphology in Megaloblastic Anemia**

#### **Erythropoiesis**

Megaloblastic changes occur at all stages of red cell development. The primitive cell is the promegaloblast, from which a series of maturing cell develop, namely basophilic, polychromatic and orthochromatic megaloblasts.

Megaloblasts differ from their normoblastic counterparts in the following respects.

Cell size: Megaloblasts are larger than erythroblasts, with an increase in cytoplasm and nuclear size at every stage of development.

Nucleus: The chromatin network is more open, being arranged in a fine reticular fashion to give a stippled appearance. Thus, the stippled appearance is commonly still well marked in polychromatic cells. The nucleus of the chromatin cell is commonly indented or lobulated, and one or more Howell Jolly bodies may be present.

Mitosis: mitosis are more common and are some times abnormal in appearance

Maturation: Megaloblastic erythropoiesis is characterized by an increase in the proportion of more primitive cells.

Prussian blue staining of the marrow shows an increase in the number and size of iron granules in erythroid precursors. Iron in reticulum cells is increased.

### **Leukopoiesis**

The characteristic feature is the presence of large atypical granulocytes, which occur at all stages of development but particularly at the metamyelocyte, resulting in (giant stab) forms. The giant stab cell has a large U-shaped nucleus, which may be irregular in outline. These cells result from asynchronism between the development of nucleus and the cytoplasm. They probably die within the marrow, and the hypersegmented neutrophils of the peripheral blood do not appear to be derived from them. The absolute number of developing granulocytes in the marrow is actually increased in nucleated red cells.

### **Megakaryocytes**

Usually are normal or slight increases. Occasionally decreased with some cells are atypical or hypersegmented nucleus.

## **PERNICIOUS ANEMIA**

Idiopathic anemia (Addison's D, Biermer), is a disease characterized by gastric parietal cell atrophy. This defect causes decreased secretion of IF and other gastric juices. The lack of IF leads to defective vitamin B12 absorption and consequently megaloblastic anemia.

Pernicious anemia is more common in people after age 50. In blacks, the disease may start earlier, with a mean of 53 years. Pernicious anemia is rare in children, and if it occurs, it may be in the congenital form. Congenital pernicious anemia is characterized by the total absence of IF and normal secretion of other gastric juices.

### **Pathophysiology**

The main cause of pernicious anemia is atrophic gastritis characterized by atrophy of the gastric mucosa with decrease of gastric secretions and IF. The cause of gastric atrophy however is not clearly known. It is postulated that genetic, immunologic, and environmental factors all play a role. IF is essential for absorption of vitamin B12. In absence of IF, only a small amount of vitamin B12 is absorbed.

The congenital form of pernicious anemias is inherited as an autosomal recessive trait. The genetic contribution to the adult form of pernicious anemia is supported by: (1) the concordant presence of pernicious anemia in identical twin (2) the increased risk in relatives of patients with pernicious anemia, (3) the presence of achlohydria with or without malabsorption in relatives of patients with pernicious anemia may produce antibody to gastric parietal cells.



The cause for the genetic predisposition of pernicious anemia is not yet clear. The association of pernicious anemia and the human leukocyte antigen (HLA) is not conclusive. Association of HLA-B7 and pernicious anemia has been reported in whites.

## Diagnosis

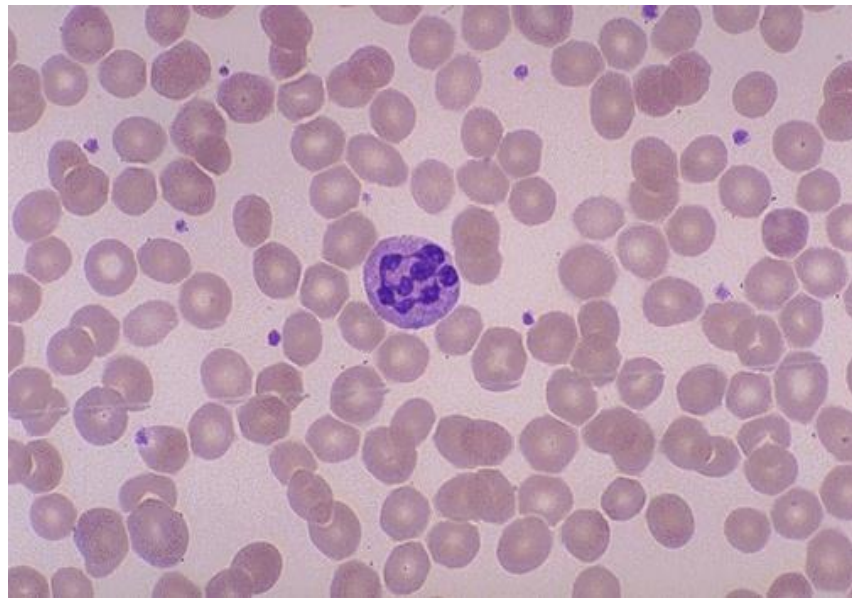
### Clinical Features

The common features are anemia (angina effort, CCF), paraesthesia, Glossitis, recurrent diarrhoea, anorexia, weight loss, abdominal pain, mental disturbance, and visual disturbance.

### Blood Picture

Hb decreased (7-9 g/l up to 3g/dl, occasionally is normal. MCV increased MCH is variable and MCHC is normal or slight increase (33-38 pg)

**Blood Smear:** Macrocytic cells many of these are oval. A small number of nucleated red cells and cell containing Howell-Jolly bodies are seen. A moderate leukopenia is associated with hypersegmented neutrophils are always present. A few myelocytes may appear in the peripheral blood. A moderate thrombocytopenia is usual with the platlet count 100000-150000/ $\mu$ L



**Figure 9.1:** Peripheral blood of megaloblastic anemia patient, showing ovalo-macrocytosis of red blood cells and hypersegmented neutrophils (8 lobes) .

### **Biochemical Finding**

The serum bilirubin is usually at the upper limit of normal but may be slightly increased.

Serum haptoglobin level is reduced. Serum ferritin and iron are elevated but fall within 48h of adequate treatment. Plasma lactate dehydrogenase is increased. The direct coombs test is positive in 10%. The serum folate is usually normal but it may be elevated or rarely reduced.

Serum vitamin B12 assay is reduced. The presence of intrinsic factor antibodies is strong evidence in favour of a diagnosis of pernicious anemia. Parietal cell antibodies are positive.

Radioactive vitamin B12 absorption test (Schilling test).

Serum Gastric Level: A pentagastrin or histamin fast achlohydria is almost inavailable in pernicious anemia, and 80% of patients have an elevated serum gastrin. The test is not specific for pernicious anemia. Reticulocytosis is response to vitamin B12 administration.

### **Treatment**

It is critical that an accurate diagnosis be made before therapy is started because folate supplementation may mask underlying B12 deficiency by improving the anemia, but not the neuralgic disease, associated with vitamin B12 deficiency and thus allowing the neuropathy to progress. Folate deficiency is usually treated with oral daily replacement (1mg/day). B12 deficiency associated with pernicious anemia requires lifelong treatment. All patients should start with intramuscularly injection therapy at 100 to 1000 µg/day, to be given every week for 1 month. Maintenance treatment may be administered intramuscularly, subcutaneously, orally, or intranasally. In general, there is a dramatic improvement in well being within a day of therapy with parental B12. Reticulocytosis becomes apparent in 1 week, whereas anemia resolves in 2 months. Neurologic symptoms take longer to improve (6-12 months). Unfortunately, in upto 10% of patients with neurologic complications, and depending on the severity and duration of disease, the damage may be irreversible.

### **Response to Therapy**

The initial sign of a positive response to therapy is an increase in the reticulocyte count. The number of circulatory reticulocytes increases 2 to 3 days after therapy with a peak at about 7 days. The reticulocyte count may increase to 50 to 70% initially. The megaloblastic morphology of the bone marrow disappears within the first 24 to 48 hours after therapy. The hematocrit rises in about 5 to 7 days after therapy, reaching normal levels in 4 to 8 weeks. Giant metamyelocytes and hypersegmented neutrophils disappear within 2 weeks. The entire therapeutic response process may take only 3 to 6 weeks depending on the severity of the disease.

## REVIEW QUESTIONS

1. The pathophysiology of megaloblastic anemia is:
  - a. Defective RNA synthesis and abnormal cytoplasm maturation
  - b. Defective DNA synthesis and abnormal nuclear maturation
  - c. Defective RNA synthesis and abnormal nuclear maturation
  - d. Defective DNA synthesis and abnormal cytoplasm maturation
2. All of the following laboratory findings coincide with megaloblastic anemia except:
  - a. Increased serum bilirubin
  - b. Increase serum iron
  - c. Decrease muramidase
  - d. Increased LDH-1
3. Megaloblastic anemia is associated with:
  - a. Ineffective erythropoiesis and increased reticulocytes
  - b. Ineffective erythropoiesis and decreased reticulocytes
  - c. Ineffective erythropoiesis and decreased LDH
  - d. Ineffective erythropoiesis and decreased erythropoiesis
4. According to the morphological classification of anemia is a:
  - a. Macrocytic, hypochromic anemia
  - b. Macrocytic hyperchromic
  - c. Macrocytic, normochromic
  - d. Normocytic, normochromic
5. Which of the following is not seen on the peripheral smear of megaloblastic anemia?
  - a. Macro-ovalocytes
  - b. Hypersegmented neutrophils
  - c. Hyposegmental neutrophils
  - d. Howell-Jolly bodies
6. Which of the following are the characteristic findings of the bone marrow in a patient with megaloblastic anemia?
  - a. Hypercellular with low M:E ratio
  - b. Hypercellular with high M:E ratio
  - c. Hypocellular with high M:E ratio
  - d. Hypocellular with low M:E ratio
7. The glycoprotein necessary for absorption of vitamin B12 is:
  - a. Albumin
  - b. Transcobalamin II
  - c. Haptocorrin
  - d. Intrinsic factor
8. All of the following are clinical manifestations of both B12 deficiency and folate deficiency except:
  - a. Anemia and jaundice
  - b. Weakness and shortness of breath
  - c. Thrombocytopenia and bleeding
  - d. Hemoglobinuria
9. Which of the following schilling test results corresponds to a diagnosis of pernicious anemia?
  - a. Part I abnormal, part II not corrected
  - b. Part I abnormal, part II corrected
  - c. Part I and part II are abnormal



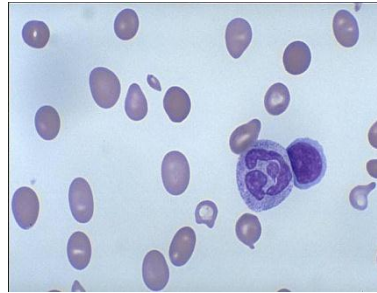
- d. Part I normal, part II corrected
10. Which of the following is not a cause of vitamin B 12 deficiencies?
- a. Atrophic gastritis
  - b. Total gastrectomy
  - c. Blind loop syndrome
  - d. Chronic gastritis
11. Hypersegmented neutrophils, a classic (nonspecific) finding in megaloblastic anemia, generally have -----or more nuclear lobes.
- a. 4
  - b. 6
  - c. 8
  - d. 10
12. Pernicious anemia is caused by a:
- a. Dietary folate deficiency
  - b. Dietary vitamin B12 deficiency
  - c. Reduced intrinsic factor secretion in the stomach.
  - d. Defective intrinsic factor molecule
13. The laboratory findings in megaloblastic anemia many include:
- a. Decrease serum folate
  - b. Decrease erythrocyte, leukocyte and platelet .
  - c. Decrease serum vitamin B12
  - d. All of the above
14. Megaloblastic changes in the peripheral blood include:
- a. Giant neutrophils with nuclear hypersegment
  - b. An MCV as high as 130 fl
  - c. Oval macrocytes with increased cetral pallor
  - d. All of the above
15. Megaloblastic changes in the bone marrow include:
- a. Giant leukocyte , especially metamyelocyte
  - b. A hypercellular marrow with leukocyte precursors predominantly
  - c. An increased ratio of erythroblasts to myeloblasts
  - d. All of the above
16. Megaloblastic anemias can be due to:
- a. Tapeworm infection
  - b. Gastric resection
  - c. Nutritional deficiency
  - d. All of the above
17. Megaloblastic anemia related to folic acid deficiency is associated with:
- a. Abnormal absorption
  - b. Increased utilization
  - c. Nutritional insufficiency
  - d. All of the above

18. The underlying gastritis that causes pernicious anemia is immunologically related to:

- a. Autantibodies to intrinsic factor
- b. A serum inhibitor of intrinsic factor
- c. Autoantibodies to parietal cells
- d. All of the above

19. The suspected blood values related to the blood film are:

- a. MCV increased, MCH increased, and MCHC normal
- b. MCV increased, MCH variable and MCHC normal
- c. MCV increased, MCH decreased, and MCHC normal
- d. MCV normal, MCH increased and MCHC normal



20. In a case of classic pernicious anemia, the patient has:

- a. Leukopenia
- b. Hypersegmented neutrophils
- c. Anemia
- d. All of the above

## **BONE MARROW FAILURE, PANCYOPENIA**

**10**

Peripheral blood cytopenia ( anemia (Hb<11g/d) , leukopenia (WBC <4000/ $\mu$ L), or thrombocytopenia (Platelet <150000/ $\mu$ L) can be due to increased loss, sequestration, consumption, or destruction of mature circulating blood elements, or to impaired production of mature cells resulting from a process that affects the origin of these cells, the bone marrow

### **Causes**

1. Aplastic anemia
2. Pancytopenia due to marrow replacement.  
This may be caused by:  
Neuroblastoma, Myelofibrosis  
Myelodysplastic syndrome, Osteopetrosis
3. Other causes:  
Systemic LE  
Paroxysmal N.H  
Overwhelming sepsis  
Hypersplenism and Megaloblastic anemia  
Hairly cell leukemia, acute leukemia, multiple myeloma and lymphoma

### **Diagnosis**

Investigations of patients with pancytopenia:

Clinical features

Blood examination

Bone marrow aspiration and trephine biopsy

- ❖ When these data do not establish the diagnosis, further investigations are necessary.

### **Blood Examination**

Presence of anisocytosis and poikilocytosis e.g acute leukemia, aplastic anemia.

Poikilocytosis is very marked in myelofibrosis.

White and red cell precursors: Relative small total nucleated red cells in myelofibrosis. A leuk-erythroblastic picture is common in subleukemic leukemic and metastatic carcinoma in bone and lymphoma.

Blast cells are common in subleukemic leukemia and acute myelofibrosis.

Immature lymphoid or plasmatic cells in lymphoma and multiple myeloma.



Marked rouleaux formation with ESR very high (>100-150/h) are common in multiple myeloma and macroglobulinemia.

Bone marrow hypercellularity in hypersplenism patients is due to active erythropoiesis and leukopoiesis.

3. Constitutional when associated with inherited defects in DNA repair as seen in Fancon's syndrome.

### **Drugs Associated With Idiosyncratic Aplastic Anemia**

1. Anticonvulsants e.g Hydantoin group
2. Antibacterial e.g Chloramphenicol, Sulphonamide, Isoniazid, Arsenical
3. Tranquilizers: Meprobamate, chlorodiazepoxide
4. Anti-Rheumatic drugs: Indomethacin, phenylbutazon
5. Antidiabetic drugs: Tolbutamide

### **Chemical Exposure**

- Benzene
- Insecticides (DDT)
- Trinitrotoluene

### **FANCONI'S ANEMIA (FA)**

FA is a familial aplastic anemia.

Onset is most common in the first decade of life.

FA is associated with patchy brown cutaneous pigmentation, neurological, renal or skeletal malformation.

Increase random chromosome breakage during mitosis with diminished capacity for DNA repair. Aberration of DNA may serve as an initiating event, the development of aplastic anemia or of leukemia.

### **Clinical Features of Aplastic Anemia**

Although the clinical onset is usually insidious, often occurring over weeks or months after exposure to a toxin, occasionally it is explosive. Signs vary with the severity of the pancytopenia.

General symptoms of anemia are usually severe. Waxy pallor of skin and mucous membrane is characteristics. Chronic cases may show considerable brown skin pigmentation.

In aplastic anemia severe thrombocytopenia may occur, with bleeding into the mucous membranes and skin. Hemorrhage into the ocular fundi is frequent. Agranulocytosis with life threatening infections is common. Splenomegaly is absent, unless induced by transfusion hemosiderosis.

The clinical presentation of pure RBC aplasia is generally milder. Symptoms relate to anemia or to the underlying disorder.

### **Blood Picture**

Hemoglobin is often as low as 7g/dl and may be considerably less. Anemia is normochromic and normocytic, although minor or moderate degree of macrocytosis are surprising common. MCV can be elevated. RBC anisocytosis is common and poikilocytosis can occur.

Reticulocyte: The absolute concentration of reticulocytes is usually depressed. The percentage of reticulocytes can be subnormal, normal or slight increased. A relatively high reticulocyte count is a good prognostic factor.

WBCs are leukopenic, particularly neutropenia. There is typically a relative lymphocytosis.

- Platelets: Thrombocytopenia. ESR; is usually elevated, some times to high values. The serum iron level is usually elevated

- The Ham's acid-serum test is occasionally positive in the absence of overt features of paroxysmal nocturnal hemoglobinuria.

### **Bone Marrow Picture**

A "dry" tap in which no material at all is obtained or a "blood" tap in which there is blood no particles can occur in this condition. In this condition bone marrow biopsy is indicated.

Cellularity: Hypocellular (in most cases)

(A). In Aplastic Particles: The proportion of fat cells increases, with a corresponding decrease in hematopoietic cells. Erythropoiesis and leukopoiesis are equally reduced or that one is relatively less affected

(B). In cellular particles:

Reduced proportion of fat cells and increased proportion of hematopoietic cells and the trails are cellular. Erythropoiesis is normoblast but often dyserythropoietic features are present particularly in the more mature erythroblasts.

Megakaryocytes; are commonly reduced in numbers even in cellular region.

The iron content is usually normal or increased.

## **MYELOYDYSPLASTIC SYNDROME (MDS)**

MDS are a heterogenous group of leukemia-related conditions characterized by various combinations of anemia, neutropenia, thrombocytopenia usually with a normocellular or hypercellular bone marrow. *Transformation to an acute myeloid leukemia occurs in some cases.*

### **Etiology**

The etiology of primary MDS is unknown. Most cases of primary MDS occur without a known exposure to a leukogenic agent. Secondary MDS can sometimes be directly related to a known agent. Examples of diseases that precede MDS include ovarian carcinoma treated with alkylating agents (10% to 15% of MDS cases), Hodgkin's disease treated with combined therapy, chemotherapy and radiotherapy. Some predisposing factors for MDS may be genetic.

**Table 10.1 : Myelodysplastic syndrome (MDS) according to FAB classification**

TYPE	BONE MARROW
1. Refractory anemia (RA)	Blasts <5%
2. Refractory anemia with ring sideroblastic (RARS)	Blast <5%
3. Refractory anemia with excess of blast (RAEB)	Ring sideroblasts >15%
4. Refractory anemia with excess blast in transformation (RAEB-t)	Blast 5-20%
5. Chronic myelomonocytic leukemia (CMML)	Blast 20-30%
	Peripheral blood monocytes increased.



**Table 10.2: WHO classification of MDS**

	Peripheral blood	Bone marrow
Refractory anemia (RA)	Anemia No blasts	< 5% blasts <15% ringed sideroblasts
RA with ringed sideroblastic (RARS)	Anemia No blasts	< 5% blasts =15% erythroid ringed sideroblasts
RA with excess blast-1 (RAEB-1)	Cytopenia <5 % blasts Absence of Auer rods	5-9% blasts Absence of Auer rods
RA with excess blast-1 (RAEB-2)	Cytopenia, <5% blasts Auer rods may be present <1000 ul monocyte	10-19% blasts Auer rods may be present
MDS, unclassified (MDS-U)	Cytopenia No blasts Absence of Auer rods	Dysplasia in granulocytes or megakaryocytes < 5% blasts Absence of Auer rods
MDS with isolated (del(5q))	Anemia <5% blasts Absence of Auer rods	Normal or increased megakaryocyte Absence of Auer rods del(5q) the only cytogenetic abnormality

**Clinical Features**

MDS is more common at age more than 50 years. It is characterized by anemia, infections which are difficult to eradicate and hemorrhagic disorders.

**Blood Picture**

Anemia is severe normocytic or mild macrocytic. Dimorphic hyponormochromic. Basophil stippling and presence of nucleated red cells. Neutropenia is common with agranular or pelger Huet anomalies. Platelets decreased

**Bone marrow**

BM is normocellular to hypocellular and dyserythropoietic and megaloblastoid changes. Multinuclearity and nuclear fragmentation are frequent. Other features of bone marrow are cytoplasmic vacuolation, Howell-Jolly bodies and ring sideroblasts in RARS.

**Calculation of Myeloblast Percentage in Bone Marrow:**

The FAB classification of ANLL (M1 to M7) and MDS necessitates the determination of the percentage of myeloblasts in bone marrow. This is particularly important in distinguishing FAB M2, FAB M6, RAEB and RAEB-T.

With formula given below, the current proposal is:

More than 30% myeloblasts and an actual percentage of erythroblasts over 50% =M6

More than 30% myeloblasts and an actual percentage of erythroblasts under 50% =M2

Twenty to 30% myeloblasts and a real percentage of erythroblasts under 50% = RAEB-t

Five to 20% myeloblasts and a real percentage of erythroblasts under 50% = RAEB

The accepted FAB standard for the calculation of the percentage of myeloblasts is accomplished by subtracting all nucleated erythroid precursors in the bone marrow from the differential count. The calculation is performed as follows:

### Example 1

1. Count all nucleated cells in the bone marrow: Total 100 cells
2. If the erythroid precursors are over 50%. Subtract the erythroid precursors (E) from the total (100) count: 100 total cells - 55 (E) = 45 cells
3. List the number of myeloblasts in the 100-cell count: Number of myeloblasts = 25 cells
4. Calculate the percentage of myeloblasts in the nonerythroid cell count. Twenty-five myeloblasts were included in the nonerythroid count of 45 cells. Therefore the percentage of myeloblasts in this count is  $(25/45) \times 100 = 55\%$

This patient has acute myelogenous leukemia, FABM6

### Example 2

100 total cells - 55(E) = 45 cells

Number of myeloblast = 10 cells

Calculation:

$(10/45) \times 100 = 22$

Note: this patient has MDS (RAED)

Table 10.3 : International Prognostic Scoring System for MDS Risk Factor Categories

Point	Bone marrow %	Karyotype	Cytopenia
0	<4	Good	0 or 1
0.5	5-10	Intermediate	2 or 3
1.0		Poor	
1.5	11-20		
2.0	21-30		
Risk level			
Low (0), Intermediate 1 (0.5-1.0), Intermediate2 (1.5-2) High =2.5			

### Treatment of MDS

Treatment consideration in MDS must weight the risk of therapy versus the risk of problems associated with existing cytopenias, and the likelihood and imminence of leukemic transformation. The risk of progression to acute leukemia ranges from 10% to 100%.

The forms of therapy for MDS include:

1. Vitamin supplementation (folate, vitamin B6 and pyridoxine in high doses, 150mg/day)
2. Blood transfusion is given when a patient has symptomatic anemia, and platelet transfusions when the platelet count falls below 10,000.
3. Erythropoietin (EPO) at 40,000 U once or twice weekly is shown to produce a response in about 15% to 20% of patients.
4. Myeloid growth factors such as granulocyte colony stimulating factor (G-CSF) either alone or in combination with EPO
5. Immunomodulators  
**Thalidomide** inhibits angiogenesis, alters cellular immune responses, modulates various cytokines, and has direct antileukemic antiproliferative effects  
Lenalidomide is a derivative of thalidomide and has a similar mechanism of action and best in patients with deletion 5 q- abnormalities
6. Demethylating agents  
Azacitidine has shown an overall response rate of 60% with a complete remission rate of 70% of patients with MDS
7. Bone marrow transplantation (Hematopoietic Stem Cell Transplantation)  
Allogeneic HSCT is the primary curative treatment for patients with MDS

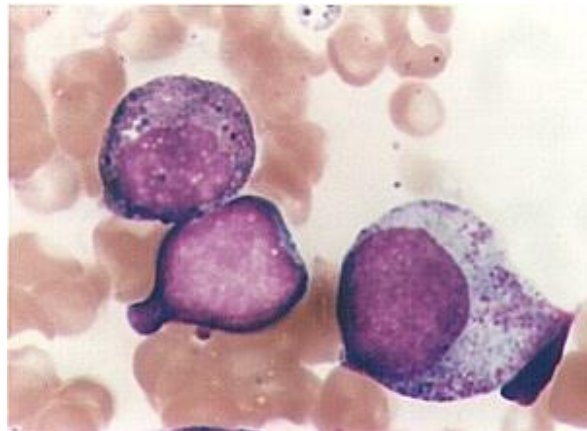


Figure 10.1: RAEB-t

#### REVIEW QUESTIONS

1. The bone marrow in aplastic anemia is characterized as
  - a. Normocellular
  - b. Having increased fibrosis in the biopsy
  - c. Having increased fat in the biopsy
  - d. All of the above
2. The peripheral blood in aplastic anemia is characterized by:
  - a. Neutropenia
  - b. Thrombocytopenia
  - c. Leukopenia
  - d. All of the above



3. Which of the following is true about Fanconi anemia?
  - A. The platelet count is normal
  - b. Patients are anemia but have a normal absolute neutrophil count
  - c. Hematological cell count abnormalities are present by 1 year of age.
  - d. The bone marrow eventually become aplastic
  
4. A leukoerythroblastic reaction is characterized by the inappropriate release from the bone marrow of
  - a. Neucleated red blood cells
  - b. Immature granulocytes
  - c. All of the above
  - d. Non of the above
  
5. Anemia associated with endocrine disorders may result from:
  - a. An increase in erythropoietin
  - b. An increase in testosterone
  - c. Hyperadrenalism
  - d. A decrease in thyroid function ( Hypothyroidism)
  
6. Which of the following terms was not used to refer to myelodysplastic syndromes?
  - a. Myeloproliferative syndromes
  - b. Refractory anemia
  - c. Smoldering leukemia
  - d. Myelodysplasia
7. Patients with some variety of MDS are at increased risk of developing:
  - a. ALL
  - b. AML
  - c. CLL
  - d. CML
8. An increased incidence of MDS is seen in :
  - a. Male less than 55 years old
  - b. Female less than 55 years old
  - c. Males more than 55 years old
  - d. Female more than 55 years old.
9. MDS and ----- can have similar clinical and morphological features.
  - a. Aplastic anemia
  - b. Iron deficiency anemia
  - c. CLL
  - d. ALL
10. Which of the following drugs is least frequently associated with aplastic anemia?
  - a. Chloramphenicol
  - b. Chloral hydrate
  - c. Phenylbutazone
  - d. Tolbutamide

# SPLEEN

# 11

## Functions of the Spleen

One of the primary functions of the spleen is filtration of defective cells. Erythrocytes experience a slow passage through the hypoxic and acidotic environment of the splenic cords and then squeeze through narrow slits into the sinusoids. Although healthy erythrocytes readily accomplish this, many aged and abnormal red cells remain behind to be ingested by the macrophages lining the cords. Abnormal cells, such as spherocytes, sickle cells, antibody-coated erythrocytes, or platelets (especially those with light coatings of immunoglobulin G [IgG]) are cleared mainly by the spleen. The spleen also is critical in clearing foreign cells, such as circulating bacteria. The amorphous polysaccharide coat of encapsulated bacteria greatly impairs their clearance in the absence of antibody; only the spleen's highly efficient phagocytic cords can effectively clear these bacteria. The splenic white pulp then processes these intravenous antigens and produces antibody that, during subsequent exposures, allows for efficient clearance by the remainder of the MPS.

The splenic cords are uniquely capable of removing erythrocytic inclusions, such as nuclear remnants (ie, Howell-Jolly bodies) or precipitated globin (ie, Heinz bodies), without destroying the cell. The spleen also serves as a reservoir for platelets and produces blood components (extramedullary hematopoiesis) if the bone marrow is unable to meet demands.

## Causes of Splenomegaly

### (a). Inflammatory splenomegaly

1. Bacterial infections: bacterial endocarditis, typhoid and paratyphoid relapsing fever, brucellosis, acute miliary Tb, secondary and congenital syphilis.
2. Viral infections: Infectious mononucleosis, viral hepatitis, primary atypical pneumonia.
3. Parasites: Kala azar, malaria, chagas disease, amoebic abscess, Bilharziasis, Echinococcosis.

### (b) Congestive splenomegaly

- Portal hypertension
- Liver cirrhosis
- Bant's syndrome

### (c) Hyperplastic splenomegaly

- Various types of hemolytic anemia
- Thrombocytopenic purpura
- Polycythemia vera, Myelosclerosis
- Leukemia, Pernicious anemia and Primary hypersplenism

- (d) Reticuloendothelial disease
- Hodgkin's disease, NHL, sarcoidosis, macroglobulinemia
- (e) Infiltrative splenomegaly
- Amyloidosis, Naumann-pick disease, Gaucher's disease
- Glycogen storage disease.
- (f) Other causes
- Felty's syndrome, Still's disease, SLE
- Tumor of spleen and acromegaly.
- (g) Infections
  1. Acute septicemia, bacterial endocarditis, typhoid, infectious mononucleosis
  2. Chronic: Tb, Brucellosis, syphilis, malaria, leishmania, schistosoma mansoni

### **Causes of Huge Spleen**

- |                             |                          |
|-----------------------------|--------------------------|
| 1. Chronic myeloid leukemia | 2. Myelofibrosis         |
| 3. Polymorphous leukemia    | 4. Amyloidosis           |
| 5. Gaucher's disease        | 6. Chronic malaria       |
| 7. Kala azar                | 8. Tropical splenomegaly |
| 9. Cooley's anemia          |                          |

### **Investigations of Splenomegaly**

#### **I. Blood Examination**

##### **(a) Red Cell Changes**

Anemia is present in leukemia, hemolytic disorders, lymphoma, thrombocytopenic purpura, hypersplenism, myelofibrosis and liver cirrhosis.

Increased red cells in Polycythemia Vera

##### **(b) Leukocytic changes**

Leukemia: leukocytosis- immature form

Infectious mononucleosis: leukocytosis, Neutropenia and abnormal lymphocytes.

Hodgkin's disease: lymphocytopenia and eosinophilia.

##### **(c) Platelet changes**

Reduced number in thrombocytopenic purpura

Increased number in thrombocythemia and early in course of CML and Polycythemia vera.

##### **(d) Pancytopenia in hypersplenism.**

(e) Blood smear for parasites as malaria, kala azar and borrelia recurrentis.

(f) Blood culture may reveal typhoid, brucella abortus and is also positive in cases of septicemia.

(g) Immunoglobulin assay in case of macroglobulinemia and heavy chain disease.

#### **II. Serological Test**

Widal test and serological test for bilharziasis, brucellosis, relapsing fever and toxoplasmosis are advised for causative diagnosis. Other tests are detection of the antinuclear antibody (ANA) and rheumatoid factor (RF).



### III. Bone Marrow Examination

This can be diagnostic in leukemia, polycythemia vera, multiple myeloma and myelosclerosis. Also it may be helpful in certain cases as Hodgkin's disease, NHL, and lipoid storage disease.

### IV. Lymphnode Biopsy: It is helpful in

1. Hodgkin's disease and NHL
2. Tb, Toxoplasmosis
3. Sarcoidosis

### V. Splenic Aspiration

### VI. Measurement of Portal Pressure and Liver Function Test

### VII. Splenic Angiography

### VIII. Splenic Scan using $^{51}\text{Cr}$ -labelled red cells or $^{99\text{m}}\text{Tc}$

### IX. $^{51}\text{Cr}$ -labelled red cells to evaluate the degree of splenic sequestration of red cell in-patient with anemia.

## HYPERSPLENISM

Hypersplenism refers to reduction of one or more of the formed blood elements (Red, white, platelets), due to overactivity of the spleen.

### Causes

1. Idiopathic
2. Secondary:
  - Portal hypertension
  - Sarcoidosis, Gaucher disease
  - Felty's syndrome
  - Kala azar, chronic malaria, Tropical splenomegaly
  - Bacterial infection, Tb
  - Thalassemia
  - CLL, Myelofibrosis, Hairy cell leukemia, lymphoma

### Clinical Features

1. Progressive anemia, which is a resistant to treatment.
2. Increased susceptibility to infection due to reduction of leukocytes such as sore throat, chest infection and vulvo-vaginitis.
3. Bleeding tendency due to thrombocytopenia
4. Splenomegaly
5. Features of underlying cause in secondary cases.

### Diagnosis

1. To establish that hypersplenism exist
2. To establish the cause of the splenomegaly

### Diagnostic Criteria

The four criteria important for the diagnosis of hypersplenism:

1. Peripheral blood picture of anemia, neutropenia and thrombocytopenia, either singly or in combination.
2. A normally cellular or hypercellular bone marrow
3. Splenomegaly

4. Significant improvement in the peripheral blood picture following splenectomy.

*The absence of a clinically palpable spleen does not absolutely exclude the diagnosis in a patient in whom the other features are suggestive.*

#### **Investigations**

1. Blood: cytopenia, anemia is normocytic normochromic with reticulocytosis, granulocytic count is markedly reduced but lymphocyte are normal or increased and platelet count is reduced.
2. Bone marrow: hyperplasia of all elements.
3. <sup>51</sup>Cr labelled red cells and platelets are useful in evaluating the degree of splenic overactivity and in predicting the outcome of splenectomy.

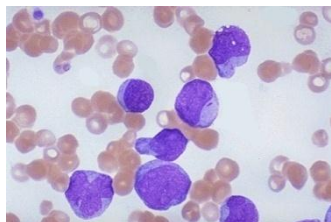
#### **Treatment**

1. of underlying cause or associated disease
2. Splenectomy-the likely benefits and possible hazards must be weighed carefully. Decisions depend on: (i) The severity of the cytopenia (2) The associated disease state (iii) The general state of the patient
3. Consequences of the splenectomy
  - i. Reduction in plasma volume to normal or near normal
  - ii. Red cells (Blood film) : Appearance of target cells, H-J bodies and anisocytes
  - iii. White cells: Early increase in neutrophils, within hours and subsequent falls towards normal with increase in lymphocytes
  - iv. Platelets- early increase, within hours and subsequent falls toward normal
  - v. Increased susceptibility to infection, notably in children.
4. Failed splenectomy-the persistent or recurrence of hypersplenism suggests an incorrect diagnosis or the presence of accessory spleens.

#### **REVIEW QUESTIONS**

1. Which of the following would not be expected to be associated with hypersplenism in adult patients?
  - a. Gaucher disease
  - b. Sickle cell anemia
  - c. Thalassemia
  - d. Chronic malaria
2. Which of the following would not be characteristic of hypersplenism?
  - a. Hypocellular bone marrow
  - b. Leukopenia
  - c. Anemia
  - d. Thrombocytopenia
  - e. Splenomegaly

## ACUTE LEUKEMIA



12

Acute leukemias are stem cell disorders characterized by a neoplastic proliferation and accumulation of immature hematopoiesis cells in the bone marrow.

### Classification

1. Acute Myelomonocytic or Myeloblastic (Non-Lymphoid)
2. Acute Lymphocytic or Lymphoblastic

In AML, the myeloblast are peroxidase and Sudan black B positive, whereas in the entire lymphoblastic are negative. The finding of Auer rods or granules in blast cells on Romanowsky stained smear will also help identify blasts of the myeloid lineage.

**Figure 12.1: Differentiation between AML and ALL.**

AML	ALL
Big people (Adult)	Small people (children)
Big blasts	Small blasts
Lots of cytoplasm	Little cytoplasm
Lots of nucleoli (3-5)	Few nucleoli (1-3)
Lots of granules and Auer rods	No granules
Big toxicity of treatment	Little toxicity of treatment
Big mortality rate	Small mortality rate
Myeloperoxidase	PAS (periodic acid schiff)

FAB-Classification: French-American-British cooperative group has classified the acute leukemia into subcategories according to the morphologic and cytochemical characteristics.

### Table 12.2 FAB-Acute leukemia classification

- M0 Myeloblastic leukemia minimally differentiated
- M1 Myeloblastic leukemia without maturation
- M2 Myeloblastic leukemia with maturation
- M2 baso Myeloblastic with basophil blasts
- M3 Hypergranular promyelocytic leukemia
- M3 variant micro or hypogranular bilobed promyelocyte
- M4 Myelomonocytic leukemia (M4 and M4 Eos)
- M5 Monocytic leukemia (M5a poorly diff and M5b Well diff)
- M6 Erythroleukemia
- M7 Acute megakaryoblastic leukemia

- ALL-L1 Small homogenous
- ALL-L2 Large heterogenous
- ALL-L3 Burkitts cell type



## ACUTE MYELOBLASTIC ( NON-LYMPHOCYTIC) LEUKEMIAS (AML)

AML occur primarily in adults and in infants less than a year old, and accounts 15% of the leukemias in children.

AML is sharp increase in adults over 50 years old.

### Pathophysiology

It is not known how a hemopoietic progenitor becomes leukemia but damage to the cells genetic programme is thought to accumulate as a result of multiple separate events.

1. Radiation: The association between radiation induced genetic damage to the hematopoietic progenitors and the development of myelodysplasia and acute leukemia is seen following nuclear disasters (e.g Hiroshima, Nagasaki, and Chernobyl)

2. Chemical drugs: Drugs and chemicals that cause bone marrow depression or aplasia are capable of producing leukemia and thus are referred to as leukemogens; some of those are chloramphenicol, phenylbutazone, arsenic-containing compounds, sulfonamides and some insecticides. Certain cytotoxic agents used in the treatment of neoplasm are likewise potentially

3. Oncogenes: Molecular studies of viruses associated with certain animal malignancies have revealed a family of viral genes known as oncogenes.

4. Proto-oncogenes: Similar viral oncogenes.

This proto-oncogene concerned with the regulation of cell growth. e.g retinoic acid receptor.

5. Genetic factors: Chromosome aberration, including aneuploidy and breakage, are demonstrated in several diseases associated with an increased incidence of ANLL. These diseases include Down's syndrome (Trisomy 21), fanconis anemia (excessive chromosome breakage), Bloom syndrome (marked chromosomal breakage and rearrangement) and D-trisomy. Congenital leukemias are usually non-lymphocytic. Studies of cases of familial leukemia are also highly supportive of the genetic etiology of acute leukemia

6. Viruses: There is no conclusive evidence that viruses are causative agents of human leukemia. However, type CRNA viruses are recognized as being the most common class of tumor viruses associated with animal leukemia and lymphoma e.g HTLV-1 may cause T-cell leukemia/Lymphoma syndrome.

It is believed that the leukemic clone originates from a single mutant progenitor cell. The mutant cell retains the ability to proliferate, but has the capacity to differentiate and mature. The target cell in the malignant transformation may be the myeloid stem cell (CFU-S) or a more mature committed stem cell.

### Common Finding

1. Pallor, fatigue and weakness from the anemia are found in almost all patients.
2. Bleeding, bruising and petechial hemorrhage caused by thrombocytopenia are also constant features of the disease.
3. Fever due to infection that fails to respond to appropriate therapy may be the first sign of leukemia.
4. Splenomegaly, hepatomegaly and lymphadenopathy occur in about 1/2 of patients at the time of diagnosis.
5. Bone tenderness in 2/3 of patients.

### Hematological Findings

Anemia is normocytic normochromic, at the time of diagnosis. Occasionally, a macrocytic anemia with hypersegmented PMNs is found but the anemia does not respond to vitamin B12 or folic acid treatment. Nucleated RBC, anisocytosis, poikilocytosis are found on the blood smear. The platelet count is moderately depressed.

Hypogranular and giant forms are commonly.

The WBC count is variable, ranging from less than 1000 to  $>100000/\mu\text{L}$ , about 50% of the patients have a normal or decreased leukocyte count at the time of diagnosis; regardless of the WBC, diagnosis of AL is suggested by the presence of blasts on the blood smear.

Blasts usually compose from 15-95% of all nucleated WBC.

When Auer rods can be found in myeloblasts, monoblasts, and occasionally in more differentiated monocytic cells.

The monocytosis frequently precedes overleukemia.

The few mature neutrophils present frequently demonstrate signs of dysplasia; pseudopelger-Huet anomaly, hypogranulation and small nuclei with hypercondensed chromatin. Eosinophils and basophils may be mildly to markedly increase.

ESR moderately or markedly increased.

Bone marrow always indicated. Morphologic typing is according to French-American-British (FAB) classification. Bone marrow is hypercellular with sheets of blasts and normal cells. In leukemia with severe peripheral leukopenia, blasts are difficult to find in the blood but are always present in abnormal amounts in the bone marrow.

Blasts most compose 30% or more of all nonerythroid-nucleated cells in the marrow to distinguish leukemia from the myelodysplastic syndromes.

Auer rods present in blasts of 50% of AML and are never found in ALL. When Auer bodies are absent, blast morphology alone does not permit distinction of myeloblasts from lymphoblasts. Cytochemistry is necessary to define the myeloid nature of the blast cell population.

Cytochemistry, including Sudan black, and or myeloperoxidase, NASDA, NASDA-F, Acid phosphatase, Acid esterase and PAS ( see table 12.2).

**Table 12.3: Cytochemical differentiation of acute leukemia**

	Peroxidase	Sudan B B	PAS	ANAE
AML-M0	(+)	(+)	-	-
AML (M1,M2)	+ to +++	+++	-	-
AMMoL (M4)	- to ++	+ to ++	-	+
AMoL (M5)	-/+	-/+	-	+++
AML (M6)	-	-	++	-
AML (M7)	-	-	-	-
ALL	-	-	++	-

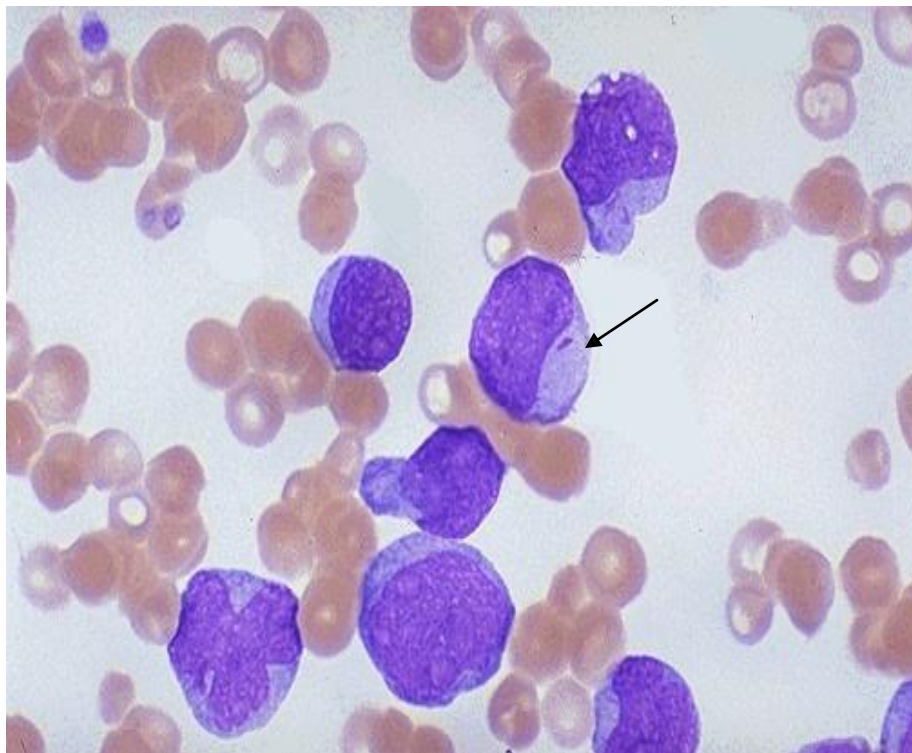


Figure 12.2: Very large immature myeloblasts with many nucleoli . A distinctive rod "AUER ROD"(→ ) composed of crystallized granules . These findings are typical for acute myelogenous leukemia (AML).



### **Immunophenotyping**

It is done on bone marrow or peripheral blood samples. The antibodies used include for ALL B lineage: CD19, CD10, CD20 and CD22. For T-lineage: CD2, CD3, CD4, CD5, CD7 and CD8. For myeloid lineage: CD13, CD33, M5 CD14 and for M6: antiglycophorin and for M7: CD 41 and CD42 and antifactor VIII.

### **Other Findings**

Hyperurecemia and an increase in LDH

Hypercalcemia with increase bone resorption, associated with leukemia proliferation in the bone marrow.

Increase serum and urine muramidase (lysozyme) are typical finding in those leukemias with a monocytic component (M4 and M5).

### **Other Specific Studies**

Karyotyping; gene rearrangement for some cases.

Electronmicroscopic studies for some cases

## **Table 12.4: World Health Organization Classification of Acute Myelogenous Leukemia (2008)**

### **AML with recurrent genetic abnormalities**

AML with t(8;21)(q22;q22); RUNX1-RUNX1T1

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11

Acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA

AML with t(9 ;11)(p22;q23); MLLT3-MLL

AML with t(6;9)(p23;q34); DEK-NUP214

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1

AML with mutated NPM1

AML with mutated CEBPA

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis

Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

## **French-American-British (FAB) Classification of Acute Myelogenous Leukemia**

The original classification scheme proposed by the French-American-British (FAB) Cooperative Group divides AML into 8 subtypes (M0 to M7)

### **AML-M0: MYELOBLASTIC LEUKEMIA MINIMALLY DIFFERENTIATED**

M0 is the most common in adult patients. Accounts for approximately 5-10% of all AML patients. WBCs show Leukocytosis in 40% and > 50% with leukocytopenia.

The diagnosis is made if less than 3% of the blasts are positive for peroxidase or the Sudan black B reaction and if the Blasts are positive for the myeloid-associated markers CD13, 14, CD15 or CD33, CD34 and negative for B or T lineage marker (CD3, CD10, CD19 and CD5). Bone marrow aspirate was hypercellular in all patients and contained a large number of leukemic blasts. Almost no mature myeloid cells were seen. The blasts were small to medium-sized round cells with an eccentric nucleus. The nucleus often had a flattened shape and was sometimes lobulated or cleaved and contained fine chromatin with several distinct nucleoli. The cytoplasm was lightly basophilic without granules. Auer rods are not found.

### **AML-M1 MYELOBLASTIC LEUKEMIA WITHOUT MATURATION**

It is found in all aged groups with highest incidence seen in adult and in infants less than a year old.

Leukocytosis in about 50% of patients at the time of diagnosis. The predominant cell in the peripheral blood is usually a poorly differentiated myeloblast with finely reticulated chromatin and prominent nucleoli.

Auer rods are found in the blast of 50% of the M1. If no evidence of granules or Auer rods is present, the blasts may resemble L2 lymphoblast.

The myeloperoxidase or Sudan black B stains are positive in more than 3% of the blasts indicating granulocytes differentiation.

PAS negative. Alpha-naphthyl acetate esterase and naphthol AS-D-Esterase are negative. About 50% of the patients will have acquired clonal chromosome aberrations in the leukemic cells. CD13, 14, 15, 33 and CD34 myeloid antigens are frequently positive in M1 leukemia. The most common cytogenetic abnormalities are: t (9; 22) (q34; q11)

### **AML- M2 MYELOBLASTIC LEUKEMIA WITH MATURATION**

The presenting symptoms for M2 AML are similar to those of the M1 type. Leukocytes increased in 50% of patients. Myeloblast can usually be found in the blood smears and may be the predominant cell type. Pseudopelger-Huet and hypogranular neutrophils being most common cells are seen in M2.

The bone marrow is hypercellular and types I and II myeloblasts make up from 30-83% of the promyelocytes to mature segmented cells. The monocytic component is less than 20%, differentiating M2 from M4. Increased basophil in some patient (M2 baso). Eosinophil may be increased.

**Cytochemistry**

Peroxidase and Sudan black B is positive. NaF does not inhibit esterase. PAS is negative. Nonspecific esterase is negative.

Positive reaction with CD13 and CD15 antigens are frequently seen in cases of M2. Some of M2 have a translocation between chromosome 8 and 21 and (q22; q22).

**AML M3: PROMYELOCYTIC LEUKEMIA**

Occur in younger adult. Median age and survival average about 18 months. M3 is of particular interest because it results in the fusion of a truncated retinoic acid receptor alpha (RAR-alpha) gene on chromosome 17 to a transcription unit called PML (for promyelocytic leukemia) on chromosome 15. It is interesting to note that high doses of the vitamin A derivative all-trans-retinoic acid are able to overcome thus block in differentiation both in vitro and in vivo and this agent has been successfully used to induce remission in patients with AML.

The most clinical finding in initial diagnosis is bleeding.

It is believed that the release of large numbers of promyelocytic granules containing a procoagulant initiate disseminated intravascular clotting (DIC). This is the most serious complication of M3 AML. Two forms of M3 have been described the typical hypergranular type, and the hypogranular or microgranular variant.

Cytochemistry: Peroxidase and Sudan black B are positive

The PAS is negative. Nonspecific esterase is negative.

Immunological studies demonstrate positivity with CD13, CD15, CD1 and CD33 myeloid antigens.

Cytogenetic studies have revealed a high prevalence (almost 50%) of the chromosomal translocation t (15; 17 associated with both AML M3 and M3m variant

**AML- M4 MYELOMONOCYTIC LEUKEMIA (Naegel, monocytic leukemia)**

It is distinguished from M1, M2, and M3 by an increased proportion of leukemia monocytic cells in the bone marrow or blood or both. Gingival hyperplasia with gingival bleeding is present.

Serum and urine levels of muramidase (lysozyme) are usually elevated because of the monocytic proliferation.

The leukocyte count is usually increased monocytic cells (monoblast, promonocytes monocytes) are increased to 5000/ $\mu$ L or more.

Anemia and thrombocytopenia are present in almost all cases.

The marrow differs from M1, M2 and M3 in those monocytic cells exceed 20% of the nonerythroid nucleated cells.

The sum of the myelocytic cells including myeloblasts, promyelocytes and later granulocytes is >20% and <80% of nonerythroid cells. This bone marrow picture together with a peripheral blood monocyte count of 5000/ $\mu$ L or more is compatible with a diagnosis of M4.

Confirmation of the monocytic component of this subgroup requires cytochemistry. The profile includes positive reactions for Sudan black B or peroxidase and both specific and non-specific esterase. A few cases of M4 AML are characterized by increased marrow eosinophils and classified as M4e.

Immunological studies demonstrate positivity with CD13, CD33, CD11b and CD14. Cytogenetic: inv (16) (p13; q22) and del (16)(q22)



### **AML-M5 MONOCYTIC LEUKEMIA (Schilling leukemia)**

Common findings: weakness, bleeding and a diffuse erythematous skin rash. There is a high frequency of extramedullary infiltration of the lungs, colon, meninges, lymphnodes, bladder and larynx.

Gingival hyperplasia

Serum and urinary muramidase levels are often extremely high.

The one criterion for a diagnosis of M5 is that 80% or more of all nonerythroid cells in the BM are monocytic cells.

There are two distinct forms 5a (maturation index <4%) and 5b (maturation index > 4%).

M5a: Granulocyte <20% and Monocyte >80% >80% monoblast

M5b: Granulocyte <20% and Monocyte >80% <80% monoblast  
(Characterized by the presence of all developmental stages of monocytes; monoblast, promonocyte, monocyte)

Non-specific esterase stains and alpha-naphthyl esterase is positive. PAS is negative.

Myeloperoxidase and Sudan black are weak diffuse activity in the monoblast.

Immunological studies demonstrate positivity with CD11b and CD14

Abnormalities of the long arm of chromosome 11 associated with either chromosome 9 or 19.

### **AML-M6 ACUTE ERYTHROLEUKEMIA (DiGuglielmo)**

M6 is a rare form of leukemia that primarily affects the peripheral cells. It is nonsexist in children. Clinical manifestations are similar other types of AML.

The most frequent presentation is bleeding.

The most dominant changes in the peripheral blood are anemia with sticking poikilocytosis and anisocytosis. Nucleated red cells demonstrate abnormal nuclear configuration. The leukocyte and platelets are usually decreased.

The diagnosis of erythroleukemia can be made when more than 50% of all nucleated bone marrow cells are erythroid and 30% or more of all remaining nonerythroid cells are type I or type II blast cells. The erythroblast is abnormal with bizarre morphologic features. Giant multilobular or multinucleated forms are common. Other features are; fragmentation, Howell-Jolly bodies, ring sideroblast, and megaloblastic changes. Dyserythropoiesis is common.

Cytochemistry: Erythroblasts are normally PAS negative. In M6, erythroblasts especially pronormoblast demonstrates coarse positivity of PAS.

## AML-M7 ACUTE MEGAKARYOBLASTIC LEUKEMIA

M7 is rare. It occurs as a leukemia transformation of CGL and MDS. Anemia and pancytopenia is characteristic at initial diagnosis.

Peripheral blood shows micromegakaryocytes and undifferentiated blasts.

Bone marrow dry tap is common.

Bone marrow biopsy show increased fibroblasts and/or increased reticulin and presence of greater than 30% blast cells

**Cytochemistry:** Peroxidase is negative, PAS +/-, Esterase +/- and positive acid phosphatase. Cytochemical positivity for  $\alpha$ -naphthyl acetate esterase reaction and negative reaction with  $\alpha$ -naphthyl butyrate esterase is unique to megakaryoblast. (Monocytes react positively with both esterase substrates).

The monoclonal antibodies that reacts with platelet glycoprotein Ib, IIb/IIIa and IIIb, using immunologic technique as well as CD41, CD42 and CD61 positivity.

Cytogenetic: Abnormalities of chromosome 21

### Other Types

Hybrid leukemias: Are leukemias with both myeloid characteristics. They may be bilineal (a mixed population of cells expressing either myeloid or lymphoid features) or biphenotypic. The bilineal hybrid leukemia may occur synchronously (at same time) or metachronously (one leukemia followed by a relapse with a different type).

### Molecular genetics

In cytogenetically normal AMLs somatic mutations of the genes FLT3, NPM1 or CEBP2 have been identified as important prognostic factors. Patients with abnormalities of the chromosomal region 11q23 representing the MLL (mixed lineage leukemia gene) fare poorly.

## Treatment of Acute Non-Lymphoblastic Leukemia

The ultimate goal in treating ANLL is to return the bone marrow to its normal state of health and function and to achieve disease-free survival for the patient. Certain factors play an important role in determining how successful therapy will be, specifically the age and pre-treatment status. The younger the patient, and the less symptomatic he or she is before treatment, the greater the chance of a significant response.

The standard treatment of acute non-lymphoblastic leukemia is a regimen consisting of cytarabin in conjunction with an anthracycline compound (e.g daunorubicin and thioguanine (TAD9 regimen). Alternatively, idarubicin or mitoxantrone may be used instead of daunorubicin, and the drug etoposide may be added. Post remission therapy involves repetition of the remission induction regimen at least twice daily, one month apart or until recovery of bone marrow function. The duration of treatment is about 3-6 months.

Bone marrow transplantation (BMT) is the best therapeutic option for patients who have achieved remission on chemotherapy. Results of BMT are generally better in the first than in later remission.

### **Induction Therapy: (for 2 courses)**

The first aim of treatment in acute leukemias is to induce a complete remission. The standard induction protocol is the combination of anthracycline with cytosine-arabinoside. In patients who do not reach complete remission, the induction should be repeated. The criteria for a complete remission are defined as <5% blasts in the bone marrow, no blasts in the peripheral blood, adequate granulocyte (>1500/ $\mu$ l) and platelet (>100,000/ $\mu$ l)

Ara-C IV	100 mg/m <sup>2</sup> /24 h	day 1 and 2	
	continuous infusion		
	100 mg/m <sup>2</sup> every 12 h	day 3 - 8	30 minutes infusion
Daunorubicin iv	60 mg/m <sup>2</sup>	day 3 - 5	

### **Consolidation: (1 -2 course2)**

A consolidation treatment is administered to patients who have reached complete remission. Two courses of consolidation are considered as standard.

Depending on the risk factors for AML, 20-25% of the patients will survive for more than 4 years. The fraction of patients who become long-term survivors increases with intensive consolidation treatment.

Consolidation chemotherapy same as in induction

### **Maintenance Therapy: (monthly for 3 years)**

M1 AD	
Ara-c sc	100 mg/m <sup>2</sup> /12 h day 1-5
Daunorubicin iv	45mg/m <sup>2</sup> /d day 3+4
M2 AT	
Ara-C	day 1-5
Thioguanine oral	100mg/m <sup>2</sup> continuous infus. day 1-5
M3 AC	
Ara-c	day 1-5
Cyclophosphamide	100 mg/m <sup>2</sup> iv day 3

**N.B** in complete remission consider Standard-dose cytarabine (100 mg/m<sup>2</sup>/day x 5-7 d x 1-2

cycles)  $\pm$  anthracycline or Consider cytarabine 1-1.5 g/m<sup>2</sup>/day x 4-6 doses x

1-2 cycles for patients with good performance status, normal renal function, normal or good karyotype but in induction failure Matched sibling HSCT or alternative donor HSCT or high dose cytarabine  $\pm$  anthracycline (daunorubicin or idarubicin), if clinical trial not available while awaiting identification of a donor or best Supportive Care



## **Treatment of relapse AML**

Age < 60 years

Early relapse (<6 months)

Clinical trial or matched sibling HSCT or alternative donor HSCT, if donor previously identified

Late relapse

Clinical trial or matched sibling HSCT or alternative donor HSCT, if donor previously identified or repeat initial successful induction regimen

Age > 60 years

In early relapse : Clinical trial (strongly preferred) or best supportive care or gemtuzumab ozogamicin

In late relapse: Clinical trial or treatment with initial successful regimen or gemtuzumab ozogamicin or best supportive care.

## **Evaluation and treatment of CNS leukemia:**

If LP positive and symptomatic at diagnosis and no focal neurologic deficits: intrathecal chemotherapy 2x/week until clear, then weekly x 4-6 weeks

If focal neurologic deficits and/or radiographic findings of chloroma causing neurologic disease: Strongly consider Intrathecal chemotherapy 2x/week until clear, then weekly x 4-6 wk

## **Supportive Therapy**

1. Prophylactic antibiotics, including antifungals, are left to the discretion of the individual institutions.

2. Growth factors may be considered in the elderly after chemotherapy is complete under certain circumstances. Note that such use may confound interpretation of the bone marrow. Patient should be off G-CSF for a minimum of 7 days before obtaining bone marrow to document remission.

3. Blood products:

Leukocyte-depleted products used for transfusion

Irradiated blood products for patients receiving immunosuppressive therapy (fludarabine, HSCT).

Transfusion thresholds-- RBCs for Hgb 8 g/dL or symptoms of anemia; platelets for patients with platelets < 10,000/mcL or with any signs of bleeding.

CMV screening for potential HSCT candidates may be considered.

4. Tumor lysis prophylaxis: hydration with diuresis, and urine alkalinization and allopurinol.

5. Clinical evidence of tumor lysis syndrome and problematic or inability to tolerate oral medication: consider rasburicase.
  6. Saline or steroid eye drops to both eyes daily for all patients undergoing high-dose cytarabine therapy until 24 h post completion of cytarabine.
  7. Patients in remission may be screened by LP, if initial WBC > 100,000/mcL or monocytic histology.
  8. Patients receiving high dose cytarabine therapy (particularly those with impaired renal function or patients > 60 years), are at risk for cerebellar toxicity. Neurologic assessments including tests for nystagmus, slurred speech, and dysmetria should be performed before each dose of cytarabine.
- In patients exhibiting rapidly rising creatinine due to tumor lysis or who develop cerebellar toxicity, high-dose cytarabine should be discontinued.
- Patients with abnormal assessments must have a cytarabine dose reduction and should not receive high-dose cytarabine as part of any subsequent therapy. (Smith GA, Damon LE, Rugo HS, et al. High-dose cytarabine dose modification reduces the incidence of neurotoxicity in patients with renal insufficiency.

In APL, If there is a high index of suspicion of APL differentiation syndrome (fever, increasing WBC > 10,000/mcL, shortness of breath, hypoxemia, pleural or pericardial effusions), close monitoring of pulmonary status is indicated, as is monitoring for fluid overload. If the patient develops pulmonary infiltrates or hypoxemia, initiate dexamethasone for 15 days (10 mg BID for 3-5 days with a taper) and consider interrupting ATRA therapy until hypoxemia resolves.

Patients with relapsed APL or with hyperleukocytosis after ATRA may be at increased risk of CNS disease.

Prophylactic intrathecal therapy (IT) is being evaluated in this group.

Management of clinical coagulopathy and overt bleeding: Aggressive platelet transfusion support to maintain platelets 50,000/mcL, and fresh frozen plasma to replace clotting factors.

Leukapheresis is not recommended in the routine management of patients with a high WBC count in APL because of the difference in leukemia biology; however, in life threatening cases with leukostasis that is not responsive to other modalities, leukapheresis can be considered with caution.

## Complication of Treatment

After the induction treatment, the patients remain granulocytopenic for at least 15-20 days and are susceptible to bacterial and fungal infections. The disturbance of coagulation and the thrombocytopenia predispose the patient to bleeding and need support with platelet concentrates and other blood products. It was shown that myeloid growth factors (granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) given after chemotherapy shorten the period of neutropenia, especially in older patients, without decreasing the rate of complete remissions. Because the use of myeloid growth factors in AML does not improve the overall prognosis, however a selective approach should be taken, giving growth factors to older patients with a high risk of neutropenia-related infection.

**Table 12.3: Empiric treatment of fever of unknown origin in a neutropenic patient**

A. Combination treatment
Broad-spectrum penicillin+aminoglycoside
Broad-spectrum cephalosporine + aminoglycoside
Broad-spectrum penicillin + broad spectrum cephalosporine
B. Single-agent treatment
Carbamapenem
Cefapim
Ceftazidium
Piperacillin/tazobactam
C. Modification if fever does not resolve within 72-96 h
Addition of aminoglycoside, glycopeptide, fluconazole, amphotericin
For example, according to the clinical and microbial situation
D. Early antimycotic treatment if pulmonary infiltrate is present
Amphotrecin B

**Table 12.4: Antiemetic regimen for chemotherapy in leukemias and lymphomas**

Regimen	Dose
A. Ondansertron + Dexamethasone	2X8 mg i.v* 10 mg i.v
B. Ondansertron	2X mg i.v*
C. Metoclopramide +/- Dexamethasone	3X8 mg/kg# 10 mg i.v

\* First dose 1 h before chemotherapy, second and third dose after 4 and 8 h, intravenously or oral.

# First dose 1 h before chemotherapy, second and third dose after 2 and 4 h i.v



### **Treatment of Relapsed or Refractory AML**

In relapsed AML, a reduction treatment with the original protocol can be considered if the duration of the first remission is more than 6 months. In other cases and in primary refractory AML, second line protocols that incorporate high-dose cytosine-arabioside, anthracycline, and other drugs are recommended. A second complete remission can be achieved in 30-50% of patients with these protocols. If an allogeneic donor has been found, the patients should be referred for BMT without delay.

### **Treatment of APL (M3)**

Earlier studies showed that even refractory cases of APL obtained complete remission with oral all-trans-retinoic acid. The treatment of APL is the first example for a differentiation treatment of a human cancer. At present the optimal treatment for patients with APL is to combine retinoids with induction chemotherapy.

### **Immunotherapy**

Immunotherapy is a technique in which antibodies are used to treat cancer. It has been used both in an attempt to increase the patient's own immunity to the leukemic cells specifically and to increase the patient's immunity non-specifically to provide an antileukemic effect.

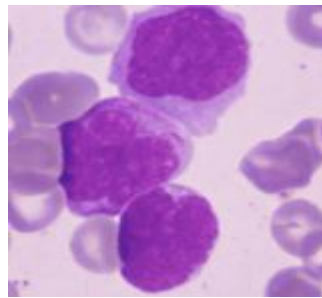
### **Bone Marrow Transplantation**

The mechanism which marrow transplantation provides an effective defence against leukemia is not well understood. To provide for transplantation, total body irradiation of 1000 rad and intensive cyclophosphamide therapy are given to destroy any residual leukemic cells.

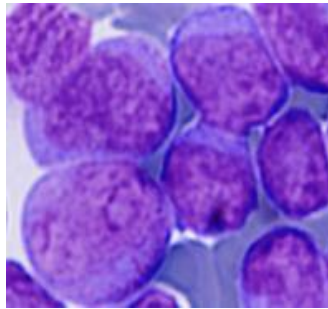
Candidates for transplantation must be in good clinical condition and in the first clinical remission for the greatest chance of success.

### **Treatment of Secondary Leukemias**

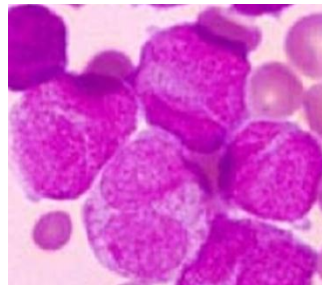
Patients with secondary leukemias have a low chance of obtaining a complete remission with current chemotherapy protocols. Therefore, these patients should undergo allogeneic BMT early if they have a histocompatible donor. The transplantation may be done without further chemotherapy or after one cycle of chemotherapy for cytoreduction to decrease the risk of relapse after transplantation.



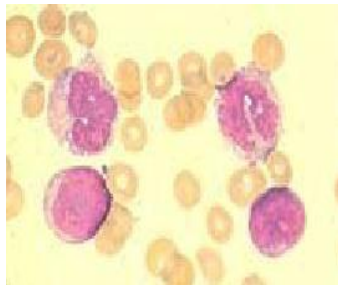
AML M1



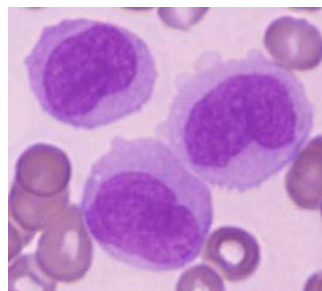
AML M2



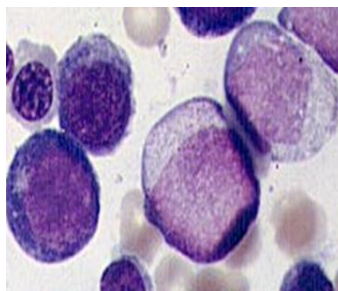
PLL M3



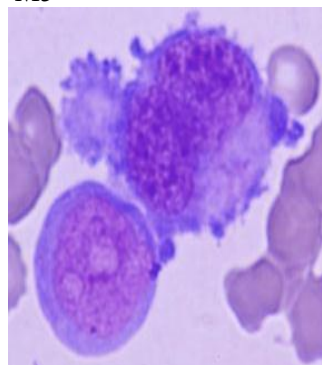
M4



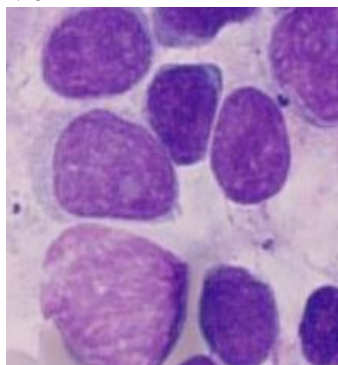
M5



M6



M7



M0

Figure 12.3: Subtype of acute myeloblastic leukemia

## **ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

ALL is malignant disease of the lymphopoietic system that is manifested by the slow but uncontrolled growth of abnormal, poorly differentiated lymphoid cells whose DNA synthesis time is significantly longer than in normal tissue.

ALL is a primary disease of young children (peak 2-5 years). The malignant lymphocytes replace normal hematopoietic tissue in the bone marrow, spleen, liver and other organs.

The predominant cell in the bone marrow and peripheral blood can be identified as a lymphoblast.

### **Etiology**

Unknown, but more common in patients with:

Immuno-deficiency

Genetic factor: Chromosomal anomalies (Down syndrome), Turner, Klinefelter

Ataxia telangiectasia, Schwachmann syndrome, Trisomy 9, 13, 21 and monosomy 7.

Leukemia in congenital disease is characterized by increased proliferation of blast cells, hyperleukocytosis and extramedullary infiltration (skin, lung)

Ionizing radiation

Exposure to electromagnetic field

Chemicals: alkylating agents

Infections: EBV

### **Pathophysiology**

The mutation of a single lymphoid stem cell is giving rise to a clone of malignant lymphocytes. These lymphoid cells retain the ability to proliferate in an unregulated manner but appear to be frozen in their maturation sequence.

### **Classification**

#### **Morphological Classification (FAB)**

ALL is divided in FAB L1 (children), L2 (older children and adult), and L3 (patients with leukemia secondary to Burkitt's lymphoma). These types are defined according to two criteria (1) the occurrence of individual cytologic features and (2) the degree of heterogeneity among the leukemic cells. These features considered are cell size, chromatin, nuclear shape, nucleoli, and degree of basophilia in the cytoplasm and the presence of cytoplasmic vacuolation.

**L1: HOMOGENOUS (Small cell):** One population of cells within the case. Small cells predominant, nuclear shape is regular with occasional cleft. Nuclear contents are rarely visible. Cytoplasm is moderately basophilic. Best prognosis. L1 accounts 70% of patients. The L1 type is the acute



leukemia that is common in childhood, with 74% of these cases occurring in children 15 years of age or younger.

**L2: HETEROGENOUS (Large cell):** Large cells with an irregular nuclear shape, cleft in the nucleus are common. One or more large nucleoli are visible. Cytoplasm varies in colour and nuclear membrane irregularities. L2 accounts 27% of ALL patients. The FAB-L2 blast may be confused with the blasts of acute myeloid leukemia. Approximately 66% of these cases of ALL in patients older than 15 years are of type 2

**L3: BURKITT'S LYMPHOMA TYPE:** Cells are large and homogenous in size, nuclear shape is round or oval. One to three prominent nucleoli and some times to 5 nucleoli are visible. Cytoplasm is deeply basophilic with vacuoles often prominent.

Patients with L3 leukemia generally have a poor prognosis because their disease responds poorly to chemotherapy.

By immunologic markers, these are B-cell malignancies. A high mitotic index is characteristic patients with L3 leukemias generally have a poor prognosis because their disease responds poorly to chemotherapy.

### **Immunological Classification**

This classification based on cell membrane markers:

1. **T-ALL:** Cells reacts with monoclonal antibodies against T-cell antigen (CD3, CD5 and CD2. About 50% of these patients have a mediastinal (thimic) mass

2. **B cell:** Cell reacts with anti-Ig reagent. And TdT is negative.

B-ALL usually corresponds to the morphological L3 type whereas the CD10+, null, pre-B or T types may all be L1 or L2 and are morphologically indistinguishable.

Burkitt's cell leukemia is positive for HLA-DR, CD9, CD22 and CD24

3. **Pre-B cells ALL:** These cells are characterized by the presence of HLA-DR, TdT, CD19, CD20 and CD24. CD10 (CALA) may be present.

4. **Common-ALL (Pro-B precursor):** The leukemic cells in this group are characterized by the presence of HLA-DR, CD10, CD19, CD24 and some time CD20. A polyclonal antibody to what is known as common acute lymphoblastic antigen (CALA), CD10 was produced by immunization or rabbits with slg and erythrocyte-rosette-negative ALL cells. CD10 is present on the leukemic cells of 70% of patients with ALL. This type accounts for approximately 85% of childhood and 75% of adult ALL. Common ALL has the highest remission rate and the longest initial remission with chemotherapy.

### **Clinical Features**

**Symptoms:** Fatigue, fever (infection), headache, nausea, vomiting.

Bone and joint pain related to the replacement of normal hematopoietic elements.

Pain in the extremities is produced by an infiltration of leukemic cells into the tissues.

### Physical Examination

#### Pallor

Evidence of hemorrhage, petechiae and also GI bleeding and hematuria.

Lymphadenopathy and hepatomegaly in 75% of patients.

Leukemic meningitis and cranial nerve palsies, headache and blurred of vision (due to nerve infiltration by leukemic blasts are quite common).

Nephropathy may be present (Lysis leukocytes after therapy).

### Laboratory Findings

Leukocytosis is common in 75% of patients and leukocytopenia in 25%

Peripheral blood smear: Blast cells in 50% of patients. The peripheral blood composed of 100% Lymphoblasts, Lymphocytes, and smudge cells.

Anemia is common due to:

- Decreased RBC production
- Blood loss
- Severe thrombocytopenia

### Bone Marrow

BM is almost always hypercellular and heavily infiltrated with or even replaced by lymphoid cells. Fibrosis is present in 10-15%. More than 30% are lymphoblast. Auer rods are not present in lymphoblasts.

### Cytochemistry

Peroxidase and Sudan black B stains are negative. PAS is Positive (coarse granular)

Acid phosphatase is positive in T-cell ALL

Chest x-ray to demonstrate mediastinal masses if present.

Bone X-ray (Skeletal survey) to detect involvement of bones

CSF examination is used for the detection of early CNS invasion.

Renal function and serum uric acid should be estimated before start of treatment.

A serious complication in ALL is infection and it is the primary cause of death in ALL. The incidence of infection is directly related to the degree of granulocytopenia.

**Table 12.5: Markers useful for subclassification of ALL**

Surface marker, protein on the cell membrane that can be detected with immunologic methods:

Type	TdT	CALLA	CD7	CD19	HLA-DR	sIg	Morphology
Common ALL	+	+	0	+	+	0	L1/L2
Pre B- ALL	+	+	0	+	+	0	L1/L2
B cell ALL	0	0	0	+	+	+	L3
T cell ALL	+	0	+	0	0	0	L1/L2

TdT - Terminal deoxynucleotidyl transferase

CALLA - common ALL antigen

sIg - surface immunoglobulin

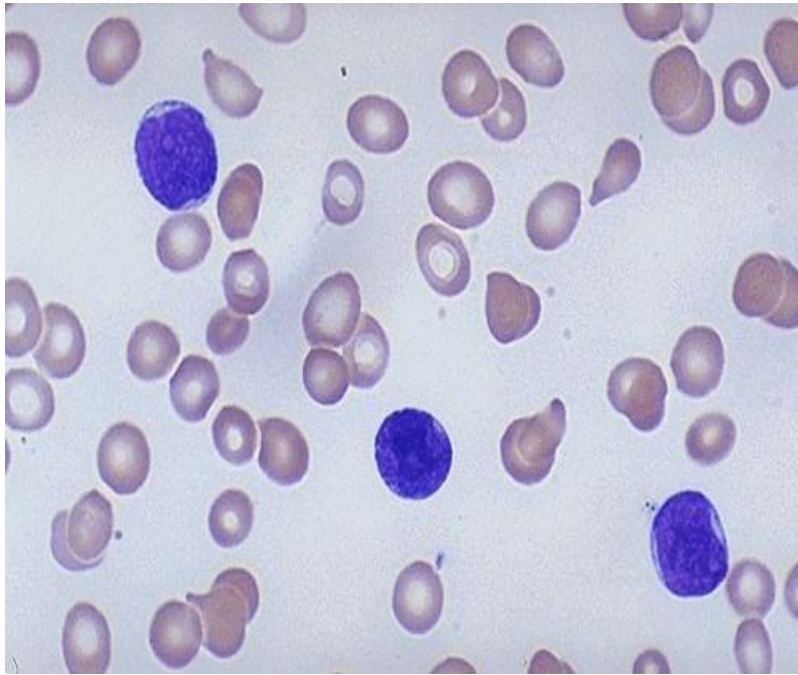


Figure 12. 4: Immature lymphoblast cells with larger nuclei that contain nucleoli . such lymphoblasts are indicative of acute lymphocytic leukemia (ALL)

### Molecular Biology of ALL

The earliest event in ALL is the transformation and clonal proliferation of a lymphoid stem cell.

With sensitive cytogenetic analysis, most cases of ALL have cytogenetic aberration (e.g translocations, deletions, inversions), some of which are prognostically relevant.

Among B-lineage AL  $t(14;18)$  is pathognomonic for the surface immunoglobulin positive B-ALL with L3 morphology (This is considered to be the leukemic form of Burkitt's lymphoma).

In B-ALL, the *c-myc* proto-oncogene is juxtaposed with immunoglobulin loci (IgH, IgK, or IgL), thereby expressing a dysregulated *c-myc* protein. In B-precursor ALL, several cytogenetic lesions are known. The  $t(1;19)$  fuses the E2A and PBX-I genes and produces a fusion protein that activates transcription through the e2a transactivation domain.

About 2-5% of children and up to 20% of adult causes with ALL have the Philadelphia chromosome ( $ph^1$ ,  $t(9; 22)$ ). Some B-precursor ALL, have abnormalities of the long arm of chromosome 11 ( $11q23$ ). In these cases the MLL gene is rearranged. In some cases of ALL, the tumor suppressor genes p15 and p16 are homogenously detected. In T-lineage ALL, a number of chromosomeal aberrations are also found. Examples are  $t(1;14)$ ,  $t(11;14)$  and  $t(7;9)$



### Detection of Minimal Residual Disease in Acute Leukemia

Relapse of leukemia following successful remission-induction therapy remains a major obstacle in the treatment of patients with acute leukemia.

Leukemia recurs most frequently in patients with acute myeloblastic leukemia (AML) and high risk acute lymphoblastic leukemia (ALL) following chemotherapy and less often in patients with low risk ALL and particularly in patient groups submitted to allogeneic marrow transplantation. It is likely that the great majority of these recurrences originate from residual leukemic cells that survive initial remission chemotherapy.

Today, several research groups throughout the world place emphasis on studies concerned with the detection and treatment of minimal residual disease "MRD". These investigations are conducted with the common objective to tackle the remaining cells.

The potential application of MRD studies in the clinical management of acute leukemia include early identification of patients at higher risk of relapse and detection of impending clinical relapse which include: (1) Measurement of early response to treatment during remission-induction (2) Identification of patients at a higher risk of relapse at the end of remission-induction early continuation (3) Detection of impending relapse and identification of leukemic cells in extramedullary sites throughout treatment. (4) Detection of contaminating leukemic cells before autologous stem cell (5) evaluation of the efficacy of purging techniques before autologous stem cell graft (6) Provide a powerful tool for assessing bone marrow or peripheral blood that has been harvested for autologous hematopoietic stem cell transplantation and (7) May serve to unequivocally demonstrate leukemic involvement of the central nervous system.

Monitoring of MRD in patients could provide useful information on the biology of acute leukemia and its responsiveness to treatment. MRD measurements could be used as endpoints to rapidly compare the effectiveness of different chemotherapeutic regimens.

Numerous methods of monitoring MRD in acute leukemia have been developed. Leukemic cells can be distinguished from normal hematopoietic cells on the basis of chromosomal or molecular abnormalities, antigen receptor gene rearrangements and immunophenotype.

To monitor residual disease, it is essential to have detailed information about the immunophenotypic features of the patients' leukemic cells at the time of diagnosis. These dictate the selection of the appropriate markers. If the immunophenotype were not known. One would have to apply the full range of potentially useful markers, an expensive and time consuming option that might still fail to identify residual disease.

The proportion of cases that can currently be monitored for MRD by flow cytometry varies from laboratory to laboratory. Factors that influence this variability include the number of markers tested; the inclusion of bone marrow sample, regenerating after chemotherapy to define the normal range, and the stringency with which the laboratory defines leukemic associated immunophenotypes. Studies with PCR now

also attempt to quantify the number of leukemic cells expressing leukemia-associated transcripts.

### **Criteria of Complete Remission**

Complete remission in clinicopathologic term includes an absolute neutrophil count of at least  $>1000/\mu\text{L}$ , a platelet count  $>100,000/\mu\text{L}$ , absence of circulating blasts, and bone marrow cellularity  $>20\%$ , with evidence of progressive maturation of all lineages and  $<5\%$  blast. The recommended criteria for the absolute neutrophil count varies from  $>1000/\mu\text{L}$  in different institutions. Detection of early relapse, like the detection of residual leukemia, is sometimes problematic. All the criteria and techniques will be described for the detection of residual disease could be used for the diagnosis of early relapse. In general, characteristic of the leukemic blast cells. However, in a small proportion of cases, changes in FAB subtype may occur. Morphologic switches between M0, M1 and M2 and between M4 and M5 are more frequent than other types of AML in relapse. Morphological changes in ALL in relapse are usually between L1 and L2, though rare cases of L1 have been reported switching to L3 in relapse. Relapse may also be associated with a change in the pattern of antigenic expression or cytogenetic findings. For example, loss or gain of CD10, TdT or HLA-DR has been described in the blast cells of relapse leukemia patients. Changes in karyotype may occur in a significant proportion of the relapsed acute leukemias.

### **High Risk Factors in ALL**

1. Age (L2 and L3 in children)  $>35$  years in adults
2. TLC  $> 30000 /\mu\text{l}$
3. Medistinal mass or lymphadenopathy
4. Immunophenotyping:
  - Unclassified non-B non T
  - Mixed lineage leukemia
5. Cytogenetic:
  - Translocation
  - Hypodiploidy
6. More than 4 weeks to complete remission.

### **Treatment of Adult Acute Lymphoblastic Leukemia**

Before the initiation of therapy, any physiologic imbalance is usually corrected. For example, correction of anemia and thrombocytopenia is accomplished by transfusion of packed red cells and platelets, antibiotic treatment is instituted for infection and intravenous supplements may be given to restore adequate hydration. Any of these measures may cause changes in cell amounts and cellular morphology.

The treatment is based on risk factors and is conducted through the following phases

In table 12. 6:

## **Table 12.6: ALL Chemotherapy**

### **1. Remission induction including**

**A). Prephase:** With one or two weeks of vincristine ( $1.4 \text{ mg/m}^2 \text{ BSA}$ ) and prednisone  $60 \text{ mg/m}^2 \text{ po}$  depending on the general condition of the patient.

This is followed by induction phase

### **B). Induction Phase: 4 Weeks**

Vincristine  $1.4 \text{ mg/m}^2 \text{ iv}$  days 1, 8, 15, 22  
Daunorubicin  $30 \text{ mg/m}^2 \text{ iv}$  day 1, 8, and 22  
Prednisone  $60 \text{ mg/m}^2 \text{ po}$  on day 1-28  
L-asparaginase  $6000 \text{ m/m}^2 \text{ sc}$  starting on day 1 of induction phase.

CNS-prophylaxis: Cranial irradiation (24Gy)

Methotrexate intrathecal,  $15 \text{ mg}$  twice weekly for a total of 5 injections.

And Hydrocortisone, cytosine arabinoside (Table 12.7)

During the cranial irradiation, the following drugs are to be given:

Cyclophosphamide ( $750 \text{ mg/m}^2 \text{ iv}$  on day 1 and 15.

6-mercaptopurine  $50 \text{ mg/m}^2 \text{ po}$  on days 15-22

### **2. Early Intensification**

This is given to patients of high-risk group. It includes 4 drugs given over a period of 4 weeks.

Vincristine  $1.4 \text{ mg/m}^2 \text{ iv}$  on day 1, 8, 15, 22  
Daunorubicin  $30 \text{ mg/m}^2 \text{ iv}$  on day 1, 8, 22  
Prednisone  $60 \text{ mg/m}^2 \text{ po}$  on day 1-28  
L-asparaginase  $6000 \text{ m/m}^2 \text{ s.c}$  every other day for a total of 10 injections.

### **3. Consolidation:** It consists of two cycles:

Vincristine  $1.4 \text{ mg/m}^2 \text{ iv}$  on day 1  
AraC  $75 \text{ mg/m}^2 \text{ s.c}$  /12hrs on days 1, 2, 3  
Cyclophosphamide  $750 \text{ mg/m}^2 \text{ iv}$  on day 1  
6-mercaptopurine  $50 \text{ mg/m}^2 \text{ po}$  on days 1-7

## **Maintenance Therapy**

### **1. Low Risk Group**

6-mercaptopurine  $100 \text{ mg/m}^2 \text{ po}$  daily  
methotrexate  $20 \text{ mg/m}^2 \text{ im}$  weekly

The maintenance therapy is interrupted every 3 months for week to give:

Vincristine  $1.4 \text{ mg/m}^2 \text{ iv}$  day 1  
Daunorubicin  $30 \text{ mg/m}^2 \text{ iv}$  day 1

Maintenance therapy continued for at least 30 months.

### **2. High Risk Group**

Allogenic or autologous Bone marrow transplantation (BMT) or maintenance therapy with the same regimen of low risk for patients who are not candidate for BMT.

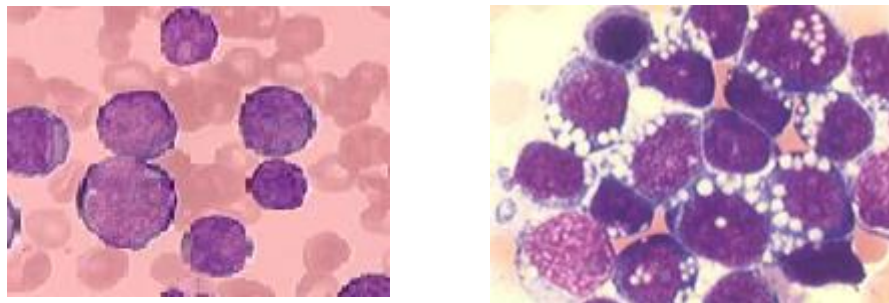


**Table 12.7: Intrathecal chemotherapy**

Drugs	Dose mg/m <sup>2</sup>
Methotrexate	15
Hydrocortisone	15
Cytosine arabinoside	30
SCHEDULE: Induction: q.wkX4; Maintenance: q.8wk 1.One year in low-risk patients and to completion of systemic therapy in high risk patients 2.Includes cranio-spinal irradiation in patients with CNS disease at onset or relapse	

### **Bone Marrow Transplantation**

The role of bone marrow transplantation in the treatment of ALL in refractory or in high-risk categories is still not established. Bone marrow transplantation should be considered in all patients who relapse and achieve a second remission, because response rate are high in ALL. Recently, stem cells harvested from peripheral blood have been used in place of marrow.



**Figure 12.5: Acute lymphoblastic leukemia (A) ALL-L2 and (B) ALL-L3**

## REVIEW QUESTIONS

1. The total blast cell count in the bone marrow is important when characterizing the acute non-lymphocytic leukemia, and must be at least.
  - a. 40% of the total nucleated cells in the marrow
  - b. 40% of the total white cell in the marrow
  - c. 30% of the total nucleated cells in the marrow
  - d. 30% of the erythroid cells in the marrow
  
2. The technique(s) used to classify the acute nonlymphocytic leukemia is/are?
  - a. Immunologic
  - b. Morphologic
  - c. Cytochemical
  - d. All of the above
  
3. All of the following stains are used to identify the acute nonlymphocytic leukemias except:
  - a. Peroxidase
  - b.  $\alpha$ -naphthyl butrate
  - c. Sudan black stain
  - d. TDT
  
4. Auer rods may be found in all of the following classification of acute nonlymphocytic leukemia except:
  - a. M0
  - b. M1
  - c. M2
  - d. M3
  
5. A cytogenetic abnormality is found in almost 50% of patients with which of the following classification of acute nonlymphocytic leukemia?
  - a. M2
  - b. M3
  - c. M5
  - d. M6
  
6. Which of the following laboratory findings would be least expected in a patient with acute leukemia at the time of presentation.
  - a. Anemia
  - b. Neutropenia
  - c. Eosinophilia
  - d. Leukocytosis
  - e. Thrombocytosis
  
7. Which of the following factors would adversely affect the ability to achieve a complete remission in an adult patient with ALL?
  - a. High white cell count at diagnosis
  - b. Presence of meningeal leukemia at diagnosis
  - c. Presence of infection at diagnosis
  - d. Presence of T-cell marker on lymphocyte

8. Which of the following FAB subgroups of AML would be expected to be characterized by intense nonspecific esterase activity in the cytoplasm?

- a. M1
- b. M2 and M6
- c. M4 and M5
- d. All of the above

9. Which of the following would be expected in acute monocytic leukemia (AmoL), but would occur infrequently in AML?

- a. Splenomegaly
- b. Hepatomegaly
- c. Gingival hypertrophy
- d. Sternal tenderness

10. Which of the following statements about the FAB classification of ALL is incorrect?

- a. It is divided into four subgroups, L1, L2, L3, L4
- b. The L1 form is the common type of childhood leukemia
- c. The L3 form is morphologically identical to Burkitt's leukemia'
- d. The L2 blasts may be confused with the blasts of acute myeloid leukemia

11. The primary cause of death in patients with ALL is :

- a. Strokes
- b. Infection
- c. Bleeding
- d. Liver failure

12. The major morphologic distinction between ALL and a reactive lymphocytosis such as infections mononucleosis (IM) is the:

- a. Difference in size of the blasts
- b. Difference in nuclear: Cytoplasmic ratio of the abnormal cells.
- c. Pleomorphic morphology among reactive lymphocytes in IM
- d. Morphologic evidence that red cells are being destroyed in IM

13. In the FAB classification of leukemias based on morphology, what percentage of cells may appear different from the proposed cell type of a specific classification?

- a. 1%
- b. 5%
- c. 10%
- d. 20%

14. The diagnosis of ALL in the adult must rule out:

- a. Leukemic lymphoma
- b. Blast transformation of CLL
- c. Acute myeloid leukemia
- d. All of the above



15. The FAB classification type of acute lymphoblastic leukemia seen most commonly in children:

- a. L0
- b. L1
- c. L2
- d. L3

16. TdT activity is present in

- a. Mature B cells
- b. Macrophages
- c. Myeloid cells
- d. Pimitive lymphoid cells

17. The lymphoblastic leukemia antigen found in 70% of patients with ALL is designated as CD:

- a. 2
- b. 10
- c. 19
- d. 22

18. The karyotype abnormality that carries a relatively good prognosis:

- a. t(8;14)
- b. t(9;22)
- c. Philadelphia ch
- d. Hyperploidy

19. Accuracy to the FAB classification of acute myelocytic leukemia, which of the following would correspond to erythroleukemia or DiGuglielmo syndrome?

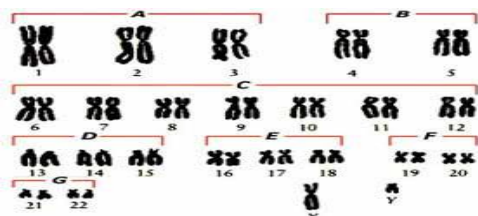
- a. M1
- b. M3
- c. M6
- d. M6

20. Which of the following would be least suggestive of meningeal leukemia?

- a. Unexplained tachycardia
- b. Isolated cranial nerve palsy
- c. Severe persistent headache
- d. Blurred vision

## CHRONIC MYELOPROLIFERATIVE DISORDERS

13



Chronic myeloproliferative disorders are neoplastic diseases of bone marrow, which affect one or more of hemopoietic elements. CMPDs usually are found in patients in their fifth and sixth decades.

Cells involved in the CMPDs include mature and immature granulocytes and platelets.

1. Chronic myeloid leukemia (CML)
2. Polycythemia Vera
3. Idiopathic myelofibrosis with myeloid metaplasia
4. Essential thrombocythemia

### Pathophysiology:

- Increased proliferation and myelofibrosis
  - Panhyperplasia of hemopoietic cells in the BM
  - Extramedullary hematopoiesis (myeloid metaplasia of liver and spleen)
- This in turn lead to transformation of this syndrome to acute leukemia, myelofibrosis and myeloid metaplasia.

**Table 13.1 Differential diagnosis of myeloproliferative disorders**

	CML	PV	IMF	ET
Age	>30	>50	>50	>50
Sex	M>F	M>F	M>F	M>F
Cell affected	Granulocyt	Erythrocyte	Fibroblast	Platelets
Peripheral blood:				
RBC ( $\times 10^{12}/l$ )	<5.5	6.5	<5.6	<5.5 - N
WBC ( $\times 10^9/l$ )	>30	<50	<50	<50 - N
Platlets( $\times 10^9/l$ )	N-Increase	N-Increase	N-Increase	>600
<u>Bone marrow</u>				
Myelopoiesis	Increase	Increase++	I-N-D	Increase++
Erythropoiesis	+++	Increase+++	I-N-D	I-N
Megakaryopoiesis	N-Decrease	Increase++	I-N-D	Increase ++
Splenomegally	N-Increase	+/-	+++	+/-
Myelofibrosis	++	+/-	+++	+/-
Philadelphia	+/-	-	-	-
ANP	+	Increase	N-I	N-I
	Decrease			

I= increase, D= decrease and N= normal

## CHRONIC GRANULOCYTIC LEUKEMIA

(Chronic myelocytic leukemia, chronic myeloid leukemia, and chronic myelogenous leukemia are synonyms for chronic granulocytic leukemia)

CML is a disease predominantly of middle life. In more than 90% of patients there are bone marrow abnormalities of the chromosome (Philadelphia chromosome-Ph<sup>1</sup>). It is a reciprocal translocation between parts of the long arms (q) of chromosome 22-G-group and C-group-chromosome 9 in nearly all instances. The resulting fusion gene (BCR-ABC) produces an altered protein believed to play a key role in the development of CML (figure 13.1).

### Etiology

Remain obscure

Epidemiological factors

1. Exposure to ionizing e.g radiologist, and in atomic bomb explosion
2. Chronic exposure to benzene

### Clinical Feature

Age 50-60 years and is possible in children.

Onset insidious

Hypermetabolic alteration: loss of weight, night sweats, loss of appetite.

Splenomegaly in most patients is gross (Huge) with pain in abdomen and under left costal margin. Splenic infarction with severe pain is common.

Anemia symptoms: Pallor, dyspnoea and tachycardia.

**Hemorrhage manifestations:** Easy bruising, nose bleeding, menorrhagia and hematomas.

Others: Gout, visual disturbance, neurological abnormalities e.g priapism (due to obstruction to blood flow in the corpus cavernosum).

### Blood Picture

Anemia is moderate with Hb 8-10 g/dl. As disease progress, the anemia becomes more severe. RBC is usually normocytic and normochromic and a small proportion of erythroblasts are occasionally present.

Leukocytes: Leukocytosis up to 500,000/ $\mu$ L or more. Segmented neutrophil and myelocyte constitutes the majority of cells. Neutrophil varies in size, giant and dwarf forms being common. Myelocytes are the characteristic cells and comprise 10-50% of the white cells. The vast majority are neutrophilic, although a few are eosinophilic and basophilic.

Myeloblast comprises up to 10%, but can rapidly increase in proportion when Blast cells occur. An increase in the proportion of basophils (2-10%) is a characteristic feature and can increase further as the disorder progresses towards transformation to acute leukemia.

The serum vitamin B12 and unsaturated vitamin B12 binding capacity are frequently increased. Uric acid is elevated. The level of serum LDH is considerably elevated in CGL.



## Cytochemistry

### Alkaline Neutrophilic phosphatase

The absence or diminution of alkaline phosphatase in the granulocytes is characteristic but not diagnostic in CML.

Normal activity is about 10-90 %

Decreased or absent in CML (0-6%), congenital hypophosphatase, paroxysmal nocturnal hemoglobinuria, sideranemia and rheumatic disease. Increased activity in: Osteomyelosclerosis, Polycythemia Vera, acute leukemia, Hodgkin's lymphoma, pernicious anemia, multiple myeloma, sepsis and carcinoma. Essential thrombocythemia, leukomoid reaction, sarcoidosis, and fever secondary to infection.

**Table 13. 2. Stages of chronic myeloid leukemia**

<b><u>A. Chronic phase</u></b> Blood GP: Pathological shift to left Pseudopelger Eosinophil increased Basophil increased EP: Normoblast occur and anisocytosis Thp: Platelet mostly increased anisocytosis. mmature platelets present	Bone marrow GP: Hyperplasia shift to left Eosinophil increased Basophil increased EP: Decreased (absolute or relative) Thp: Megakaryocytes most increased, with abnormal form
<b><u>B. Accelerated phase</u></b> Gp: Pathological shift to left with blast <20% Basophils increased <30% Ep: Normoblasts anisocytosis, polychromasia Thp: Platelets normal or decreased with anisocytosis	Gp:P pathological shift to left with blast 15-29% Basophils increased Ep: Decreased Thp: Normal or decreased
<b><u>C. Acute phase (Blast crises)</u></b> Gp: Blast cells increased Ep: Anisocytos. polychromasia Normoblast Thp Decreased or absent with anisocytosis	Gp:Blast cells increased >30% Ep: Decreased Thp: Decreased
<p><i>NB: in most cases, the blast cells are myeloid in type, but in 20-30% the blast cells are lymphoblastic cells.</i></p> <p><i>WHO criteria of accelerated phase CML include at least on of the following: 10%-19 % blasts in peripheral blood or bone marrow, 20% peripheral basophils, thrombocytopenia &lt; 100,000ul, lack response to therapy, increase spleen size and increasing WBC unresponsive to therapy, and evidence of clonal evolution.</i></p>	

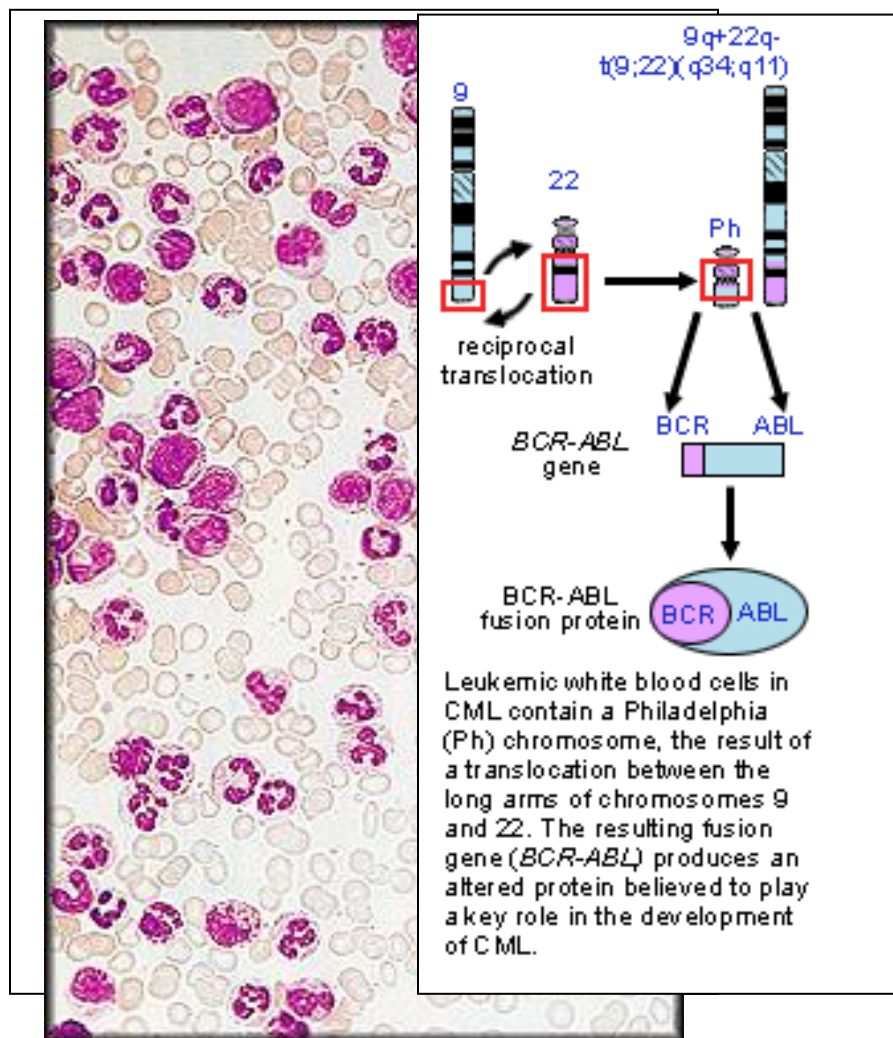


Figure 13.1: (right ) chronic Myeloid leukemia ,  
(left) philadelphia chromosome (ph)

### Bone Marrow

Marrow aspiration yields hypercellularity fragments. The cell trails are hypercellular. The cells are mainly of the myeloid series, the myelocyte being the predominant cell, although promyelocytes and myeloblasts are also increased. There is shift to left

Erythropoiesis: is normoblastic, but sometimes dyserythrocytic.

Myeloid: Erythroid ratio (M:E) is increased due to white cell hyperplasia and in the later stages there may be an actual reduction in erythropoietic tissue.

Megakaryocytes: Are often prominent and are usually smaller than normal.



## Chromosome Finding

Cytogenetic studies of bone marrow and peripheral blood cells showed that, the Philadelphia chromosome is characteristically associated with the neoplastic cells in CML.

The Philadelphia chromosome usually cannot be detected in the peripheral blood when the immature cells have disappeared, but it persists in the bone marrow cells. 92% of  $ph^1$ -positive patients have the typical  $t(9;22)$ , the remainder have variant translocations.  $Ph^1$ -negative CML represents a heterogeneous group of myeloproliferative or MDS and perhaps should not be called CML.

75%-80% of patients in a blast crisis of CML develop other chromosome aberrations in addition to the  $ph^1$  chromosome. The most common abnormalities are a duplication of the  $ph^1$  chromosome and trisomy 8.

## Treatment of Chronic Myeloid Leukemia

Special additional work up is required in CML including; cytogenetic for Philadelphia chromosome and other abnormalities, serum vitamin B12 and serum calcium. It is also important to determine the type of crisis (lymphoid or myeloid) by peroxidase and PAS.

Accelerated phase	Definition
ELN criteria	Blasts in blood or marrow 15-29%, or blasts plus promyelocytes in blood or marrow >30%, with blasts <30% Basophils in blood $\geq 20\%$ Persistent thrombocytopenia ( $<100 \times 10^9/L$ ) unrelated to therapy Clonal chromosome abnormalities in $Ph^+$ cells (CCA/ $Ph^+$ ), major route, on treatment
WHO criteria	Blasts in blood or marrow 10-19% Basophils in blood $\geq 20\%$ Persistent thrombocytopenia ( $<100 \times 10^9/L$ ) unrelated to therapy CCA/ $Ph^+$ on treatment Thrombocytosis ( $>1000 \times 10^9/L$ ) unresponsive to therapy Increasing spleen size and increasing white blood cell count unresponsive to therapy
Blast phase	
ELN criteria	Blasts in blood or marrow $\geq 30\%$ Extramedullary blast proliferation, apart from spleen
WHO criteria	Blasts in blood or marrow $\geq 20\%$ Extramedullary blast proliferation, apart from spleen Large foci or clusters of blasts in the bone marrow biopsy



## Classical Treatment of CML

A) Hydroxyurea: it is the drug available in poor world it is inhibiting the DNA synthesis. Start therapy with 2 g/day (40 mg/kg) and continue therapy for 1-4 weeks. Stop the drug when the TLC is in normal level.

B) There are five drugs currently used to target tyrosine kinase enzymes for CML, called tyrosine kinase inhibitors or TKIs: imatinib (Gleevec), dasatinib (Sprycel), and nilotinib (Tasigna), bosutinib (Bosulif), and ponatinib (Iclusig). All five drugs can stop the BCR-ABL enzyme from working, which causes the CML cells to die quickly

**Gleevec :** Imatinib is a targeted tyrosine kinase inhibitor, which antagonizes the activity of the ABL tyrosine kinase, as well as c-kit and platelet -derived growth factor alpha and beta.

Increased risk of progression to accelerated and blast phase has been demonstrated if imatinib does not help patients achieve specific clinical goals. These include :

- 1) 3 months complete haematological response with normal peripheral counts and >1 log reduction in BCR-ABL
- (2) 6 months: cytogenetic response with <35% ph chromosome positive bone marrow cell.
- (3). 12 months: complete cytogenetic response with undetectable ph chromosome
- (4) 18 months: major molecular remission with a log reduction by a qPCR of peripheral BCR-ABL.

Follow-up during initial treatment requires CBC monthly, peripheral BCR-ABL qPCR every 3 months, and bone marrow biopsy every 6 months until major molecular remission is achieved. Once molecular remission is documented , BCR-ABL transcripts should still be followed every 3 months, with an annual bone marrow exam for cytogenetics.

Resistance to imatinib has been noted in 2%-4% of patients annually for the first 3 years of imatinib therapy and may decrease thereafter.

Imatinib resistance can be overcome with either increasing doses or a second-line tyrosine kinase inhibitor such as dasatinib.

Imatinib has been shown to improve survival in accelerated phase at higher doses (600 to 800 mg), but response are often short-lived.

**Dasatinib.** In 2010, dasatinib was approved by the FDA as an initial treatment for patients with newly diagnosed chronic phase CML. It is a pill that may be taken once or twice a day depending on the dose. The side effects include anemia, neutropenia (low levels of white blood cells), thrombocytopenia (low platelet counts), and the build-up of fluid around the lungs or heart.

**Nilotinib.** Nilotinib was also approved by the FDA in 2010 as an initial treatment for patients with newly diagnosed chronic phase CML.

**Bosutinib.** In 2012, bosutinib was approved by the FDA to treat CML when one of the other tyrosine kinase inhibitor drugs was not effective or if a patient experienced too many side effects.

**Ponatinib.** Ponatinib was also approved by the FDA in 2012 for patients when one of the other tyrosine kinase inhibitor drugs was not effective or if a patient experienced too many side effects.

**Table 5. Definition of the response to TKIs (any TKI) as first-line treatment**

	Optimal	Warning	Failure
Baseline	NA	High risk Or CCA/Ph+, major route	NA
3 mo	BCR-ABL1 $\leq 10\%$ and/or Ph+ $\leq 35\%$	BCR-ABL1 $> 10\%$ and/or Ph+ 36-95%	Non-CHR and/or Ph+ $> 95\%$
6 mo	BCR-ABL1 $< 1\%$ and/or Ph+ 0	BCR-ABL1 1-10% and/or Ph+ 1-35%	BCR-ABL1 $> 10\%$ and/or Ph+ $> 35\%$
12 mo	BCR-ABL1 $\leq 0.1\%$	BCR-ABL1 $> 0.1-1\%$	BCR-ABL1 $> 1\%$ and/or Ph+ $> 0$
Then, and at any time	BCR-ABL1 $\leq 0.1\%$	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Confirmed loss of MMR* Mutations CCA/Ph+

Modifies from European Leukemia Net

(c). Allogenic hematopoietic stem cell transplant from either related or unrelated donors remain the only known curative therapy for CML. Transplantation from a matched sibling donor during chronic phase is associated with a 10-year survival of 50% to 70%

Allopurinol is usually given before therapy, coupled with adequate hydration to ameliorate the hyperurecemia caused by the rapid turnover and destruction of granulocytes by chemotherapy.

The patient in the accelerated phase during hydroxyurea treatment is given busulfan 2 mg po daily, 6-mercaptopurine 300 mg po daily and allopurinol 300 mg po daily.

The treatment of patient (non-gleevec) in acute blastic crisis varies accordingly to the type of crisis

(a) LYMPHOBLASTIC TYPE: Vincristine 1.4 mg/m<sup>2</sup> /day

Prednisone 40 mg/m<sup>2</sup> po daily 1-4

(b) MYELOID TYPE: Low dose Ara-C 10 mg/m<sup>2</sup> 12hours sc day 1-14

## POLYCYTHEMIA RUBRA VERA

Polycythemia rubra vera is a myeloproliferative disease in which there is autonomously increased activity of erythropoiesis with varying degrees of granulopoietic and megakariopoietic proliferation.

### Clinical Features

1. Plethoric appearance affecting the face, neck, hands and feet.  
The skin shows characteristic bluish red flush-ruber. A common complaint is intense itching specially after hot water baths.
2. Symptoms of low circulation
  - (a) Neurological: Headache, dizziness, vertigo, visual disturbance, paraesthesia.
  - (b) Gastrointestinal: Constipation, symptoms of peptic ulcer and may be bleeding.
  - (c) Cardiac: Dyspnea, palpitation, angina and even heart failure. The systolic pressure is elevated.
3. Symptoms of increased metabolism: sweating, weight loss.
4. Bleeding tendency due to thrombocytosis; epistaxis, bleeding gums and ecchymosis.
5. Spleen is enlarged in 75% of cases.
6. Liver is enlarged in 50%
7. Complications: Thrombosis, Gout, and Peptic ulcer, CML, Myelosclerosis.

### Laboratory Findings

1. Increased of RBC, Hemoglobin Hematocrit and blood viscosity. RBCs show microcytic, hypochromic with anisocytosis and poikilocytosis, reflecting exhaustion of iron stores due to increased haemoglobin synthesis.
2. Leukocyte counts can go as high as 40 to 50X10<sup>9</sup>/L. Occasionally, a slight shift with a few metamyelocytes is seen. Basophil numbers frequently are elevated.
3. Increased platelet count above 500000/ $\mu$ L. A moderate number of abnormal platelet forms may be present. Abnormal platelet aggregation and adhesiveness, as well as decreased level of platelet forms (PF3) are frequently seen.
4. Reduced ESR is usual.
5. Bone marrow hypercellular with panhyperplasia particularly erythroid series. Reduced iron stores and increased reticulin. It is not diagnostic, but biopsy is frequently performed to evaluate fibrosis and cytogenetics even when the diagnosis is not in question.
6. Leukocyte alkaline phosphatase score (LAP) is high.
7. Biochemistry: Vitamin B12 and B12 binding proteins are elevated. Serum erythropoietin, ferritin and folate are commonly low. Hyperurecemia is common and LDH may be elevated due to ineffective erythropoiesis.



**Table 13.3 Diagnostic criteria for diagnosis of Polycythemia**

JAK2 positive	
Hct >52% in male	
Hct > 48% in female	
JAK2 negative	
Major	
4 major or 3 major + 2 minor	
Hct > 60% (male) or >56% (female)	
No secondary erythrocytosis	
Palpable splenomegaly	
Acquired genetic abnormalities (excluding JAK2 and BCR-ABL)	
Minor	
Platelets > 450X10 <sup>6</sup> /ml	
Neutrophils >10x10 <sup>6</sup> /ml	
Radiographic splenomegaly	
Low serum erythropoietin	

## Treatment

The aim of treatment is to keep the PCV below 55% and to reduce the thrombocytosis to prevent thrombosis and hemorrhage.

A. Low-risk patients are < 60 years old and have no history of thrombosis, no cardiovascular risk factors, and platelet counts <1500X10<sup>9</sup>/L. These patients are managed with phlebotomy to a Hct of <45% and low-dose aspirin. Iron deficiency via phlebotomy is a goal of treatment.

B. High-risk patients are >60 years old or have a history of a thrombotic event, or cardiovascular risk factors, or platelet counts >1500x10<sup>9</sup>/L. These patients typically require cytoreductive agents in addition to phlebotomy and aspirin is usually held off until platelet counts are <1500x10<sup>9</sup>/L.

C. Treatment for intermediate risk patients must be individualized, as data are sufficient to clearly support either a conservative (low risk) or an aggressive (high risk) treatment plan.

1. Venesection (phlebotomy). About 0.5 - 1 litre blood is removed weekly until the PCV (hematocrit) reaches 45% and venesection is repeated whenever it approaches 55%. The average maintenance is 500 ml every 2-3 months. Also restrict iron rich diet.

2. Radioactive phosphorous is effective and has the advantage of prolonged control.

3-5 millicuries I.V and can be repeated after 10 - 12 weeks if response is not satisfactory.

3. Interferon alpha decreases both the red cell number and the frequency of thrombo-hemorrhagic events.

4. A popular drug for the treatment of PV is hydroxyurea. Normal red blood counts were found in more than 80% of patients studied within 12 weeks of starting hydroxyurea treatment. Hydroxyurea also appears to be less leukomogenic; therefore, it is the therapy of choice.

5. The treatment of complications as gout, thrombosis, and hemorrhage.

## IDIOPATHIC MYELOFIBROSIS

Idiopathic myelofibrosis is referred to as Agnogenic Myeloid Metaplasia. It is a chronic MPD of unknown cause, characterized by systemic bone marrow fibrosis and extramedullary hematopoiesis.

Secondary myelofibrosis is caused by infiltrative disorders including malignancies and infections, or exposure to chemical toxins, or irradiation. Idiopathic myelofibrosis is uncommon.

The process of fibrosis ensues from the proliferation of fibroblasts and increased collagen production in reaction to the abnormal clone of hematopoietic cells. Dysmegakaryocytopoiesis leading to an overproduction of defective platelets is the most constant features of myelofibrosis.

IM is not associated with a specific or unique chromosomal anomaly.

### Clinical Feature

It is characterized by progressive anemia; splenomegaly and fibrosis. The hepatosplenomegaly is due to extramedullary hematopoiesis. Easy bruising, resulting from thrombocytopenia or abnormal platelet function or both. More than 40% of patients suffer from osteosclerosis with accompanying bone pain and malaise.

### Hematological Finding

Leukocytosis with leukoerythroblastic picture of teardrop shaped erythrocyte, an occasional nucleated erythrocytes and immature myeloid cells is classic for myelofibrosis.

The bone marrow is hypocellular and becomes fibrotic with an associated decrease in hematopoiesis.

### Treatment

Researchers are working to develop medications that target the JAK2 gene mutation that's thought to be responsible for myelofibrosis. The first of these medications is ruxolitinib (Jakafi).

#### Treatments for anemia

If myelofibrosis is causing severe anemia, you may consider treatment, such as:

**Blood transfusions.** In severe anemia, periodic blood transfusions can increase red blood cell count

**Androgen therapy.** Taking a synthetic version of the male hormone androgen may promote red blood cell production and may improve severe anemia in some people.

**Thalidomide and related medications.** Thalidomide (Thalomid) and the related drug lenalidomide (Revlimid) may help improve blood cell counts and may also relieve an enlarged spleen.

**Surgical removal of the spleen (splenectomy).** If the size of your spleen becomes painful and begins to cause harmful complications.

**Chemotherapy.** Chemotherapy drugs may reduce the size of an enlarged spleen and relieve related symptoms, such as pain.

**Radiation therapy.** Radiation uses high-powered beams, such as X-rays, to kill cells. Radiation therapy can help reduce the size of the spleen, when surgical removal isn't an option.

**Stem cells transplant**

Allogeneic stem cell transplantation — stem cell transplantation from a suitable donor — is the only treatment that has the potential to cure myelofibrosis.

## **ESSENTIAL THROMBOCYTHEMIA**

Essential or primary thrombocythemia (thrombocytosis) is characterized by a marked increase in circulating platelets, usually in excess of  $1000000/\mu\text{L}$ . The diagnosis of ET is difficult and relies in the exclusion of other myeloproliferative states and non-hematological illness associated with an increased concentration of platelets. ET affects male and female equally, frequently among persons in the fifth and sixth decades.

Thrombotic or bleeding problems are the most commonly seen disorders in patients with thrombocythemia.

Patients typically manifest easy bruising, nose bleeding, or gastrointestinal bleeding.

### **Laboratory Finding**

The classic laboratory finding in E.T is a markedly elevated peripheral blood platelet count. The number of platelets in the circulation blood is usually in excess of  $1000000/\mu\text{L}$  with a minimum of  $600000/\mu\text{L}$ .

Hb decreased with hypochromic microcytic anemia. If splenic atrophy is present, abnormal erythrocyte morphology includes target cells, H-J bodies, nucleated erythrocytes and acanthocytes. The total concentration of WBC is elevated in about 50% of patients. The ALP value is normal or increased, concentration of vitamin B12 and uric acid are usually increased.

### **Platelet Function**

In patients with thrombocythemia, the mean extent of aggregation induced by epinephrine, collagen, or ADP is significantly lower than in normal controls.

### **Bone Marrow**

Morphology is similar to the architecture seen in polycythemia vera and CML with associated extreme thrombocytosis. Increased marrow cellularity, megakaryocytic hyperplasia is strikingly. This conspicuous megakaryocytic proliferation is also manifest polyploid of the nuclei, giant forms and clusters.

### **Treatment**

The goal of treatment patients focus on maintaining platelet count  $<600 \times 10^9/\text{L}$  and limiting thrombohemorrhagic risk. Thrombotic risk has been linked to age  $>60$  year old, prior thrombosis, and platelet count  $>400 \times 10^9/\text{L}$  to  $600 \times 10^9/\text{L}$ . Other risk factors that are still emerging and



being validated include elevated leukocytes, positive JAK2 V617F mutation, and cardiovascular risk factors.

Plateletpheresis can dramatically and swiftly reduce the platelet count. Plateletpheresis alone is not sufficient because it may stimulate thrombopoiesis and formation of blood clot. Alkalating agents and radioactive phosphorus ( $^{32}\text{P}$ ) are effective treatment.

Hydroxyurea is effective. If hydroxyurea is ineffective, Busulfan can be used with caution. Another randomized study in which high-risk patients (previous thrombosis, age  $>60$  y or platelets  $>1000 \times 10^9/\text{L}$ ) were randomized to receive hydroxyurea plus aspirin or anagrelide plus aspirin.

Symptomatic like ASA, Allopurinol, cimetidine, cyprohepatidine.

Avoid splenectomy because it can markedly increase the platelet count and the risk of both hemorrhagic and thrombotic events.

### REVIEW QUESTION

1. The best description of polycythemia vera is that it is characterized by:
  - a. Increased red cell mass
  - b. Leukopenia
  - c. Thrombocytopenia
  - d. Increased myeloblasts
2. An RBC poikilocytes that is considered to be the first sign of spent phase of polycythemia is the
  - a. Dacrocyte
  - b. Spherocyte
  - c. Target cell
  - d. Schistocyte
3. -----are the cells most responsible for the appearance of the marrow in agnogenic myeloid metaphase
  - a. Neutrophils
  - b. Erythrocytes
  - c. Lymphocytes
  - d. Fibrocytes
4. Hydroxyurea treatment may result in megaloblastic morphology because hydroxyurea is an:
  - a. Alkylating agent that damages DNA
  - b. Inhibitor of DNA replication
  - c. Inhibitor of platelet function
  - d. Inhibitor of maturation
5. Which of the following laboratory abnormalities would be least likely in a patient with PV at the time of diagnosis?
  - a. Leukocytosis with absolute granulocytosis
  - b. Normoblasts in the peripheral blood
  - c. Thrombocytopenia
  - d. Abnormal platelet function studies

6. Which criteria must be present for a diagnosis of PV that will meet the criteria of the polycythemia vera study group?

- a. Any two from category A combined with any two from category B
- b. One from category a and three from category B
- c. Elevated red cell mass and normal arterial oxygen saturation combined with any two category B criteria
- d. Non of the above

7. Which of the following is induced among the category B criteria of the polycythemia vera study group for the diagnosis of PV?

- a. Thrombocytopenia
- b. Leukopenia
- c. Elevated serum B12 or unbound B12 binding capacity
- d. Decreased leukocyte alkaline phosphatase score.

8. Which of the following would be least likely in a patient with idiopathic myelofibrosis:

- a. Tear-drop RBCs on peripheral smear
- b. Erythrocytosis
- c. Splenomegaly
- d. Nucleated RBCs on peripheral smear.

9. Which of the following is most helpful in differentiating idiopathic myelofibrosis from myelofibrosis secondary to some other process?

- a. Serum protein electrophoresis
- b. Serum uric acid
- c. Bone marrow biopsy
- d. Urinary muramidase

10. Which of the following has been most closely associated with the development of chronic myeloid leukemia?

- a. Exposure to ionizing radiation
- b. Recurrent herpes virus infection
- c. Chronic active hepatitis
- d. Lead toxicity

11. The following statement are correct about chronic myeloid leukemia except

- a. It is the commonest form of leukemia worldwide
- b. it is associated with a median survival of <2 years
- c. Often presents asymptotically
- d. It is more commonly derived from B cells than T cells

12. The following statements are correct about Polycythemia vera except:

- a. Occur more frequently in smokers
- b. May present as gout
- c. May transform to acute leukemia
- d. Is associated with an enlarged spleen

## **CHRONIC LYMPHOPROLIFERATIVE DISORDERS**

**14**

### **CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)**

CLL is the proliferation and accumulation of lymphocytes (usually B cells) that are relatively unresponsive to antigenic stimuli. CLL is a disease predominantly of the middle and older age group with 90% of the patients being older than 50 years of age and nearly 65% older than 60. Male are affected twice as frequently as females.

The lymphocytes in CLL are immunologically incompetent.

#### **Clinical Features**

##### **Common Features**

1. Symmetrical lymph node enlargement in most of the patients
2. Symptoms and signs of anemia

An important complication in approximately 10% of cases is acquired hemolytic anemia. This is sometimes the first manifestation of chronic lymphatic leukemia. It should be suspected when the degree of anemia is inappropriately severe for the degree of lymph node and splenic enlargement, the degree of lymphocytosis, or when spherocytes or agglutination are present in the blood film.

##### **Occasional Features**

3. Spleen and liver enlargement.
4. Hemorrhagic manifestation in patients with thrombocytopenia.

The causes of thrombocytopenia are like ITP. This type of thrombocytopenia is responsive to corticosteroid or splenectomy.

Impaired platelet production due to hemopoietic tissue replacement by the diseases or from myelosuppressive effects of agents used for therapy of the disorder.

5. Respiratory and other infections

Caused by:

- Impaired Ig production
  - Neutropenia
  - Corticosteroid administration.
6. Skin infiltration
  7. Tonsillar enlargement.
  8. Nervous system manifestations are due to N.S. infiltration
  9. Bone or Joint pain.
  10. Disturbance of vision or hearing.



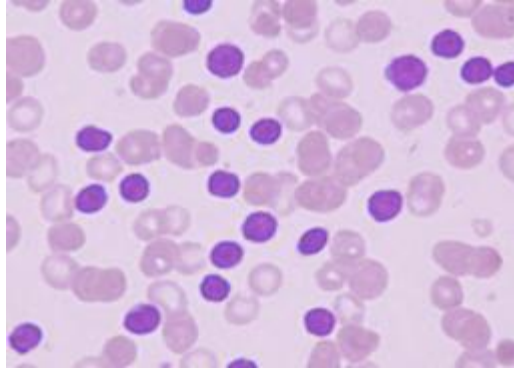


Figure 14.1:  
Chronic lymphatic leukemia

### Blood Picture

Hb is normal in early stages to moderate or severely depressed values in advanced CLL.

Anemia is usually normochromic and normocytic. When anemia is due to hemolysis, it usually has the typical features of autoantibody-mediated red cell destruction, with spherocytosis, a positive Coomb's test, and a reticulocytosis.

### Typical Feature of CLL

Leukocytosis, with lymphocytes count is ranged from 50,000 to 200,000/ $\mu$ L following statement is correct about although it is occasionally greater. Sometimes leukocytes are less than 10,000. 90% or more of the leukocytes are mature lymphocytes: Lymphocytes are usually seen as:

- (i) Small cells with densely clumped nuclear chromatin and a narrow rim of cytoplasm (predominant)
- (ii) Larger cells with lighter cytoplasm
- (iii) Smear or "basket" cells -bare lymphocyte nuclei

Neutrophils: Normal in early stage. When it become depressed as a consequence of:

- 1. Replacement of normal hemopoietic by disorder.
- 2. Hypersplenic effects.
- 3. Myelosuppression by cytotoxic therapy.

Thrombocytopenia, with counts less than 50,000/ $\mu$ L is common. It is a feature of advanced stage. Serum Immunoglobulin decreased in most CLL in late stages. It may fall to 0.3 to 0.4 g/dl and patient become more susceptible to all types of infection.

### Bone Marrow Aspiration and Biopsy

Usually are not necessary for making the diagnosis of CLL except in those aleukemic or subleukemic cases with no nodal or splenic involvement and few or no abnormal cells in the peripheral blood.

In marrow there is increase of lymphocytes and a corresponding reduction of megakaryocytes, myeloid precursors, and erythroid precursors.

Chromosomal abnormalities are detected in more than 50% of patients with CLL and indicate a worse prognosis. The most frequently encountered is trisomy 12 (+ 12). Other cytogenetic abnormalities are 14q translocation.

In more than 90% of the cases, CLL lymphocytes express the CD5 antigen, which was formerly thought to be a T-cell antigen. Cells from most cases of B-CLL also express CD19, CD24, CD37 and CD21 antigen. About 60% of CLL are positive for CD23 but infrequently demonstrate positivity for CD22

Important molecular markers include ZAP70 and IgVH gene rearrangements

**Table 14.1: The Rai staging for chronic lymphatic leukemia**

Stages	Characteristics	Survival
0	Peripheral blood lymphocytosis >15000/ $\mu$ L	>150 months
I	Lymphocytosis and lymphadenopathy	100 months
II	Hepatomegaly or splenomegaly or both	71 months
III	Anemia (<11g/dl or Hct <33%)	19 months
IV	Thrombocytopenia (platelets < 100,000/ $\mu$ L)	19 months
Adapted from Rai KR, et al, Clinical staging of chronic Lymphocytic leukemia. Blood 1975; 46:219		

## PROLYMPHOCYTIC LEUKEMIA

This is a rare form of lymphocytic leukemia in which the circulating lymphoid cells are larger and less mature in appearance than lymphocytes in chronic lymphatic leukemia.

Occur more often in elderly males and associated with splenomegaly (splenic tumour), but not particularly with lymphadenopathy.

The lymphocyte count in the peripheral blood can be greatly increased, as high as 200,000/ $\mu$ L and the abnormal cell population in most cases has a surface phenotype of B cell.

The prognosis for PLL is considerably poorer than for either CLL or HCL. The mean survival is reported to be less than 1 year.

## HAIRY CELL LEUKEMIA

HCL is a lymphoproliferative syndrome characterised by the presence in the bone marrow, spleen and the peripheral blood of mononuclear with hairy cytoplasmic projection, which can be detected by phase contrast microscope.

### Epidemiology

Incidence is 2 to 5% of leukemias. 2-3/1000000 per year

Sex: M/F 4:1, Age 40-60 years

### Etiological Factors

Unknown

Ionizing, radiation and chemicals are considered as etiological factors

### Clinical Pictures

Men are more often affected (4:1 to 5:1) with the median age at diagnosis being 55 years. Virtually none younger than 20 is affected. General signs are asthenia and general weakness

Tumoral syndrome includes hepatosplenomegaly, bone infiltration and peripheral adenopathy.

### Complications

1. Infections: Severe respiratory tract infections, Tb, Mycosis, Toxoplasmosis and Histoplasmosis
2. Hemorrhagic manifestation
3. Splenic rupture

### Blood Picture

Pancytopenia is the most consistent laboratory observation. Anemia is normocytic normochromic. Granulocytopenia and monocytopenia are the most common cause of the leukopenia seen in HCL. Cytopenia result from infiltration of the marrow with malignant cells and fibrous tissue, and the effect is augmented by sequestration of blood cells by an enlarged spleen. Platelet is moderately decreased.

The proportion of hairy cells varies widely, but is usually in the order of 10-50%

Diagnosis by bone marrow aspiration is some time difficult (Dry Tap), but trephine biopsies of bone marrow shows characteristic appearance mild fibrous and diffuse cellular infiltrate of the hairy cells varies, but most cases belong to the B-lymphocyte lineage.



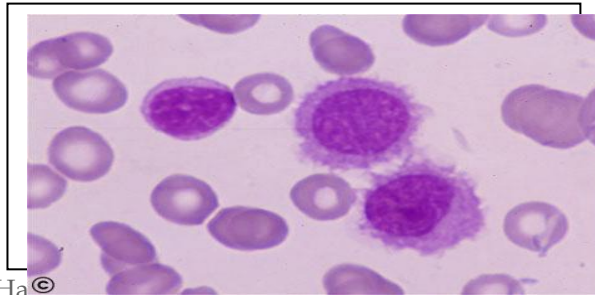
### Cytochemistry

Tartrate resistance and acid phosphatase are positive

Immunophenotyping is characteristics;

CD 25, CD 11c , CD 19, CD22 and CD103 are positive in most cases.

Further diagnostic method is by electronic microscope.



Hairy cell leukemia variant (HCL-v), which shares some characteristics with B cell prolymphocytic leukemia (B-PLL), does not show CD25 (also called the Interleukin-2 receptor, alpha).

### Treatment of Chronic Lymphoproliferative Disorders

Chronic lymphocytic leukemia (CLL) is classified in to 5 immunologic subtypes, namely

1. B-cell CLL
2. B-cell prolymphocytic leukemia (PLL)
3. T-cell CLL
4. T-cell prolymphocytic leukemia
5. Hairy cell leukemia

The CLL patients are staged according to Rai staging system. Most patients are managed supportively. Asymptomatic patient and those with early stages (0,I, II) may not be treated. 2/3 of patients respond to therapy with a single alkylating agent such as chlorambucil which may be used (20-30 mg/m<sup>2</sup> orally every 2-4 weeks; or in a daily dose of 2-5 mg/m<sup>2</sup> until WBCs stabilized. Patients with stage III and IV are treated with chlorambucil combined with prednisone (20-40mg/m<sup>2</sup>/d) for one week every 2-3 weeks. Prednisolone is effective in the treatment of autoimmune hemolysis or thrombocytopenia.

Purine Nucleoside Analogues: (E.g deoxycytosine, 2-chlorodeoxyadenosine, fludarabine) based on analogues of adenosine have recently become available for the treatment of CLL and low grade lymphomas. Fludarabine may be more effective as a single agent than chlorambucil. Fludarabine also useful in patients resist to chlorambucil (25-30 mg/m<sup>2</sup>/day i.v for 45 days) repeated each month for 3-6 months.

Alternatively, they may be given VCP regimen every 3 weeks:

Vincristine            1.4mg/m<sup>2</sup> iv day 1

Prednisone 600mg/m<sup>2</sup> orally day 1-5

Current therapies of CLL include purine analogues (fludarabine and cladribine), monoclonal antibodies against CD20 (rituximab) and CD52 (alemtuzumab), radiation, and alkylating agents (chlorambucil and cyclophosphamide). FCR (Rituximab 375 mg/m<sup>2</sup> IV day 1, Fludarabine 25 mg/m<sup>2</sup> IV days 2-4, Cyclophosphamide 250 mg/m<sup>2</sup> IV over 1 hour days 2-4 ) have shown response rate of 30% to 60% in fludarabin-pretreated populations. Alemtuzumab is showing increasing promise as a single or combined agent in refractory disease.

**Autologous and allogenic bone marrow transplantations are being explored as treatment options.**

#### **Other Measures**

**Cyclosporin:** may be useful in red cell aplasia.

**Immunoglobulin** replacement: e.g. 250-mg/kg month by intravenous infusion is useful for patients who have hypogammaglobulinemia and/ or recurrent infections and a poor IgG immune response to pneumococcal polysaccharide vaccination.

Prophylaxis against pneumocystis, herpes simplex virus, and varicella zoster virus, as well as a monitoring for CMV reactivation should be considered when treating CLL patients with these agents.

#### **Prolymphocytic leukemia**

Therapy: Polychemotherapy (e.g CHOP)

Splenectomy is usually of benefit and Purine nucleoside may help.

#### **Hairy cell leukemia**

If the patient is asymptomatic, no therapy is needed. In the symptomatic patients, chemotherapy has not been very effective. Aggressive therapy with splenectomy is one method of treatment, especially in those patients with severe cytopenias which are attributable to splenic sequestration of cells. Hairy cell leukemia is especially problematic in those patients who do not improve after splenectomy. Successful treatment with interferons (IFN $\alpha$ ) has been reported .

Cladribine (2CDA) and pentostatin (DCF) are the two most common first-line therapies. They both belong to a class of medications called purine analogs.

The most common treatment for cladribine-resistant disease is infusing monoclonal antibodies that destroy cancerous B cells. Rituximab is by far the most commonly used.

## **Differential Diagnosis with Leukemia Leukemoid Reaction**

The term leukemoid reaction is used to describe the occurrence of a peripheral blood picture resembling that of leukemia, because of marked elevation of the total white cells count, or the presence of immature white cells, or both. Leukemoid reactions may be either myeloid or lymphoid.

### **Myeloid Leukemoid Reaction**

Increased of total leukocytes count  $> 50000/\mu\text{L}$  and myelocytes and/or myeloblasts appear in the peripheral blood.

#### **Causes**

1. Severe infections
2. Secondary to nonhematological malignancy
3. Acute hemolysis.

#### **Clinical Feature**

Features related to the causative disorder.

#### **Laboratory Finding**

Leukocytes are usually moderately increased but not exceeds  $100000/\mu\text{L}$   
Myelocyte 5-15% and blasts  $< 5\%$ . Toxic granulation and Doehle-bodies may be seen

Anemia may occur. Plateles are normal to increase, but reduced in leuko-erythroblastic anemia and intravascular coagulation.

Bone marrow: White cell hyperplasia may be present with normal immature cells.

ALP increased (in CML is decreased).

Absence of Philadelphia chromosome

### **Lymphatic Leukemoid Reaction**

#### **Causes**

1. Infectious mononucleosis
2. Cytomegalovirus
3. Pertussis, measles, chickenpox
4. Tuberculosis
5. Carcinoma

### **Leukoerythroblastic Reaction**

Presence of immature myeloid cells and nucleated RBC in the peripheral blood.

#### **Causes**

1. Secondary carcinoma of bone
2. Myelofibrosis, Thalassemia major, Hemolytic anemia, Multiple myeloma, Lymphoma, Gaucher's and Niemann-pick disease, Marble bone disease.

#### **Blood Film**

Anisocytosis, Poikilocytosis (Tearshaped), nucleated RBC (10% of WBC) Reticulocytes  $> 3\%$ -10%) is usual.



Leukocyte cells show shift to left (Total N-increase but some times decreased). Platelet count is normal or reduced.

**Table 14.1 Features of leukemoid reaction and leukemia**

Features	Leukemoid reaction	Leukemia
Clinical	Evidence of infection	Hepatosplenomegaly Lymphadenopathy
Hematology	No anemia No thrombocytopenia	Anemia present Thrombocytopenia present
Bone marrow	Normal, hypercellular	Presence of leukemic blasts Decreased megakaryocytes Decreased erythroid precursors
Leukocyte alkaline phosphatase	High	Absent

*\*Philip Lanzkowsk: Pediatric Hematology and Oncology*

## REVIEW QUESTIONS

### 1. Chronic lymphocytic leukemia

- Often gives positive results with TRAP stain
- Commonly demonstrates CD5 positivity and trisomy 12
- May only be diagnosed by bone marrow examination
- All of the above.

### 2. Prolymphocytic leukemia

- It is characterized by massive lymphadenopathy and hepatosplenomegaly
- It is characterized by almost total bone marrow replacement by prolymphocytes.
- Has a comparable prognosis to CLL and HCL.
- All of the above

### 3. Hairy cell leukemia is a disease in which the abnormal cells

- All have a scanty amount of cytoplasm
- Demonstrate positivity with tatarate-resistant acid phosphatase stain
- Excess the CD5 surface marker
- All of the above

### 4. Which of the following could cause a patient to be stage VI in the Rai system?

- Bone marrow with greater than 40% lymphocytes
- Platelet count less than 100,000
- Hemoglobin less than 11g/dl
- Presence of splenomegaly
- All of the above

5. Which of the following would least likely be associated with lymphocytosis?
- Cytomegalovirus infection
  - Pneumococcal pneumonia
  - Infectious mononucleosis
  - Tuberculosis
6. Which of the following would be most useful in differentiating CLL from infectious mononucleosis?
- Presence of immune hemolytic anemia
  - Splenomegaly
  - Cervical lymphadenopathy
  - Lymphocyte morphology
7. Which of the following would be an indication for splenectomy in a patient with CLL?
- Immune thrombocytopenia controlled only with large steroid.
  - Pancytopenia with increased hematopoietic marrow elements and splenomegaly.
  - Immune hemolytic anemia controlled only with high dose steroid.
  - All of the above
8. Which of the following could be expected in a patient with Hairy cell leukemia?
- Occur primarily in infants and children
  - Splenic enlargement is uncommon
  - Fever without infections is frequently encountered
  - Granulocytosis is the most frequent WBC abnormality.
9. Which of the following statements about the cytotoxicity drug therapy of CLL is true?
- Busulfan (Myeleran) is considered the drug of choice.
  - Vincristine (oncovin) is best avoided because it produces thrombocytopenia.
  - Cyclophosphamide is effective only when used in combination with prednisolone
  - About 2/3 of patients respond to therapy with a single alkylating agent.
10. Which of the following is the best description of chronic lymphocytic leukemia?
- A disease which often transforms into ALL
  - A disease in which immunologically incompetent B-cell accumulate
  - A disease which is treated with Busulfan for symptoms control
  - A disease etiologically linked to radiation exposure.

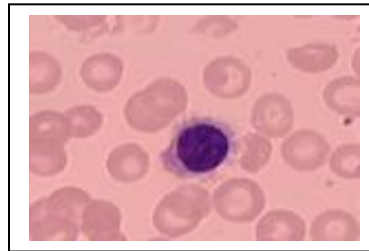
11. All are true about CLL except:
- a. Is a cause of hypogammaglobulinemia?
  - b. Is commonly treated with intensive combination chemotherapy
  - c. Often presents asymptotically
  - d. Is more commonly derived from B cells than T cells

12. The following statement are related to chronic lymphatic leukemia except
- a. CLL is slowly progressive, with good short-term but poor long term survival
  - b. CLL may be complicated by autoimmune hemolytic anemia
  - c. The Philadelphia chromosome is the sine qua non of CLL
  - d. Most patients are managed supportively

**13. QUIZ**

*What do you think that leukocyte in this smear is related to :*

- a. Chronic lymphocytic leukemia
- b. Prolymphocytic leukemia
- c. Hairy cell leukemia
- d. Se'zary syndrome





Lymphomas are a group of malignant neoplasms characterized by the proliferation of cell native to the lymphoid tissues, i.e. lymphocytes, histiocytes and their precursors and derivatives.

## Classification

1. Non-Hodgkin's Lymphoma
2. Hodgkin's lymphoma

## NON-HODGKIN'S LYMPHOMAS (NHL)

A heterogenous group of malignant diseases arising from different immune cell types principally T and B-lymphocytes.

It is a localized or generalized lymphadenopathy. In about 1/3 of cases, it may be primary in other sites where lymphoid tissue is found, e.g. in oropharyngeal region, gut, bone marrow and skin. Lymph node enlargement due to lymphomatous disease must be differentiated from that caused by the more frequent infectious and inflammatory disorders.

Lymphomatous involvement often produces marked nodal enlargement, which is almost always nontender. Although variable, all forms of lymphoma have the potential to spread from their origin in a single node or chain of nodes to other nodes, and eventually to disseminate to the spleen, liver and bone marrow. Some, often becoming widespread, spill over into the blood, creating leukemia like picture in the peripheral blood.

## Etiology and Risk Factors

**Chromosomal translocations** and molecular rearrangements nonrandom chromosomal and molecular rearrangements play an important role in the pathogenesis of many lymphomas and correlate with histology and immunophenotype. The most commonly associated chromosomal abnormality in NHL is the t(14;18)(q32;q21) translocation, which is found in 85% of follicular lymphomas and 28% of higher-grade NHLs. This translocation results in the juxtaposition of the *bcl-2* apoptotic inhibitor "oncogene" at chromosome band 18q21 to the heavy-chain region of the immunoglobulin locus within chromosome band 14q32.

The t(11;14)(q13;q32) translocation results in overexpression of *bcl-1* (cyclin D1/PRAD 1), a cell-cycle-control gene on chromosome 11q13, and has a diagnostic, nonrandom association with mantle cell lymphoma.

Chromosomal translocations involving 8q24 lead to *c-myc* deregulation and are frequently seen in high-grade small noncleaved lymphomas

(Burkitt's and non-Burkitt's types), including those associated with HIV infection.

**Environmental factors** also may play a role in the development of NHL.

*Occupations* Certain workers have a slightly increased risk of developing NHL, including farmers, pesticide applicators (Khat and vegetables), grain (flour) millers, meat workers, wood and forestry workers, chemists, painters, mechanics, machinists, printers, and workers in the petroleum, rubber, plastics, and synthetics industries.

*Chemicals* that have been linked to the development of NHL include a variety of pesticides and herbicides (2,4-D-organophosphates, chlorophenols), solvents and organic chemicals (benzene, carbon tetrachloride), wood preservatives, dusts (wood, cotton), and some components in hair dye.

*Chemotherapy and radiotherapy* Patients who receive cancer chemotherapy and/or radiation therapy are also at increased risk of developing NHL.

**Viruses** Several viruses have been implicated in the pathogenesis of NHL, including EBV, HTLV-1, Kaposi's sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8, or HHV-8), and hepatitis C virus (HCV).

EBV is a DNA virus that has been associated with Burkitt's lymphoma, particularly in endemic areas of Africa; Hodgkin's disease; lymphomas in immunocompromised patients (ie, organ transplantation and HIV infection); sinonasal lymphoma (Asia and South America); and sporadically in other B- and T-cell lymphomas. EBV can transform lymphocytes in culture. B-lymphocytes from normal EBV-positive subjects grow as tumors in mice with severe combined immunodeficiency.

HTLV-1 is a human retrovirus that is endemic in certain areas of Japan and the A minority (5%) of carriers develop adult T-cell leukemia/lymphoma. An HTLV-1-like deleted provirus has been detected in some patients with mycosis fungoides, although conflicting findings have been reported.

KSHV KSHV-like DNA sequences are frequently detected in body cavity-based lymphomas in patients with HIV infection and in those with multicentric Castleman's disease.

HCV infection is associated with the development of clonal B-cell expansions and certain subtypes of NHL, particularly in the setting of essential (type II) mixed cryoglobulinemia. HCV may predispose B-cells to malignant transformation by enhancing signal transduction upon binding to the CD81 molecule.

**Immunodeficiency** Patients with congenital and acquired states of immunosuppression are at increased risk for NHL.

*Congenital immunodeficiency* states that are associated with an increased risk include ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia, X-linked lymphoproliferative syndrome, and severe combined immunodeficiency.

*Acquired immunodeficiency* states, such as HIV infection, iatrogenic immunosuppression (ie, organ or bone marrow transplant [BMT] recipients, long-term survivors of Hodgkin's disease), and a variety of collagen vascular and autoimmune diseases (eg, Sjögren's syndrome,

rheumatoid vasculitis and Felty's syndrome, systemic lupus erythematosus, chronic lymphocytic thyroiditis, and angioimmunoblastic lymphadenopathy) also pose an increased risk of developing NHL.

**GI lymphomas** An increased incidence of GI lymphomas is seen in patients with celiac (nontropical) sprue and inflammatory bowel disease, particularly Crohn's disease. Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is seen most frequently, but not exclusively, in association with *Helicobacter pylori* infection.

### Recognition and Classification

Six major pathological classification systems exist for NHL and these can be translated by using the National Cancer Institute Working Formulation developed in 1982 and the Revised European American Lymphoma classification in 1994.

1. British national lymphoma classification
2. Rappaport classification. 1966
3. Lukes and Collins classification 1972
4. Dorfman classification 1975
5. Kiel classification 1978
6. WHO classification

**Table 15.1: Classification of malignant lymphomas**

WORKING FORMULATION	RAPPAPORT CLASSIFICATION	IMMUNOPHENOTYPE
Low-Grade		
A. Small lymphocyte	Lymphocytic, well differentiated lymphocytic	mainly B
B. Follicular, predominantly small cleaved	Nodular, poorly differentiated lymphocytic	B
C. Follicular, mixed small cleaved and large cell	Nodular, mixed lymphocytic histiocytic	B
INTERMEDIATE-GRADE		
D. Follicular predominantly large cell	Nodular, histiocytic noncleaved	Mature B
E. Diffuse, small cleaved cell	Diffuse, poorly differentiated lymphocytic	Mature B or T
F. Diffuse, mixed small and large cell	Diffuse mixed lymphocytic and histiocytic	Mature B or T
G. Diffuse, large cell	Diffuse histiocytic	B or T
HIGH-GRADE		
H. Large cell, immunoblastic	Diffuse histiocytic	B or T
I. Lymphoblastic	Lymphoblastic lymphoma	T
J. Small noncleaved cell	Undifferentiated, Burkitt's and non-Burkitt's	B

**The Rappaport** is based on the pattern of tumor growth with the affected lymph node and the morphology of the predominant cell. The advantages of the Rappaport system are its simplicity and the fact that the different classes show some correlation with prognosis.

**The Lukes Collines** System attempted to combine immunological and morphological observation. Thus, the malignant cells are classed as B lymphoid, T lymphoid or histiocytic and related on morphological grounds to the stage of differentiation of their supposed normal counterparts.

**The Kiel system** also relies on a combined immunological and morphological approach and attempts to relate the malignant cell in each class of NHL to a normal counterpart.



### **Working Formulation for Clinical Use**

NHLs are divided into three major prognostic groupings: low, intermediate, and high grades based on survival statistics. The five-year survival rate for tumors classified as low grade from 50-70% and for tumors of intermediate and high grade from 35-45% and 23 to 32% respectively. The working formulation also contains a miscellaneous group that includes the rare histologic tumors

- The HTLV-1 induced T-cell leukemia/lymphoma
- T-Lymphomatous of skin

### **Low-Grade Lymphomas**

The low-grade lymphomas typically affect patients between the ages of 45 and 60 years. These are slow-growing lymphomas, and lymphnode enlargement can be present for years before diagnosis. Often patients can do well without treatment for 2 to 3 years.

This category includes the following tumors:

1. Small lymphocytic lymphoma
2. Follicular, predominantly small-cleaved cell lymphoma
3. Follicular mixed (small cleaved and large cell) lymphoma.
4. Marginal zone lymphoma
5. MALT lymphoma (mucosa-associated lymphoid tissue)
6. Nodular monocytoid lymphoma
7. Primary splenic lymphoma

### **Intermediate Grade Lymphomas**

This type affects individuals in late middle age. Patients present with a much rapid lymphnode enlargement, and extranodal disease is more common. The tumors under this category of the Working Formulation are:

1. Follicular, Predominantly Large Cell Lymphoma
2. Diffuse Small Cleaved Cell Lymphoma
3. Diffuse Mixed Small and Large Cell Lymphoma
4. Diffuse Large Cell Lymphoma
5. Lympho-Epithelioid Lymphoma
6. Angiocentric Lymphoma
7. Adult T-Cell Leukemia/Lymphoma

### **High Grade Lymphomas**

The high-grade lymphomas cause the most rapid enlargement of the lymphonodes and the fastest developing malignancies. Thus, if left untreated, these lymphomas are rapidly fatal. This category includes the following tumors:

1. Large Cell Immunoblastic Lymphomas
2. Lymphoblastic Lymphomas
3. Small Noncleaved Lymphoma, which include
  - Burkitt's lymphoma
  - Burkitt's like lymphoma

### **1. Large Cell Immunoblastic Lymphoma**

These lymphomas display a wide range of morphologic features.

In some cases; the tumor cells have plasmacytoid features and are therefore considered to be B Immunoblasts. These cells are 4-5 times larger than small lymphocytes have a round or oval large nucleus with 1 or 2 centrally placed nucleoli. In other cases; the tumor cells may contain large multilobated nuclei or the nucleus may be round with a clear cytoplasm.

It is of interest to note that approximately 50% of B-Immunoblastic lymphomas are associated with a previous history of an immunologic disorder such as Sjogren's syndrome, Hashimoto's and AIDS. These tumors may arise from T or B cells, but numerically most are of B-cell origin. These can be demonstrated by Immunophenotyping or analyzing Ig gene rearrangements.

### **2. Lymphoblastic Lymphoma (LL)**

This is a distinct clinicopathologic entity closely related to T-cell acute lymphoblastic leukemia (T-ALL). The tumor cell resembles the lymphoblasts of ALL. They are fairly uniform in size, with scanty cytoplasm and nuclei that are somewhat larger than those of small lymphocytes. Nucleoli are either absent or inconspicuous.

In keeping with its aggressive growth, the tumor shows a high rate of mitosis. A very characteristic clinical feature is the presence of a prominent mediastinal mass in 50-70% of patients at the time of diagnosis, suggesting a thymic origin. Indeed, the phenotype of the tumor cells resembles intrathymic T cells. (TdT) Terminal deoxynucleotidyl transferase an enzyme associated with primitive lymphoid cells is expressed in all cases. In some patients the cells are CD2+, CD5+ and CD7 as are early thymocytes, where as in others CD4 and CD8 are coexpressed on tumor cells.

### **3. Small Noncleaved Cell Lymphomas**

Within this category fall Burkitt's lymphoma and related tumors seen outside Africa.

Burkitt's lymphoma was described initially in Africa, where it is endemic in some parts, but it also occurs sporadically in nonendemic areas.

Histologically: The African and nonendemic cases of Burkitt's lymphoma are identical, although there are some clinical and virologic differences. There is relation of these disorders to the Epstein-Barr virus (EBV).

These tumors consist of a sea of strikingly monotonous cells, 10-15µm in diameter with round or oval nuclei containing 2-5 prominent nucleoli. A mitotic index is very characteristic.

In African cases, involvement of the maxilla or mandible is the common mode of presentation, whereas abdominal tumors are more common in cases seen in America.

Leukemic transformation of Burkitt's lymphoma is uncommon especially Africa cases.

These tumors respond well to aggressive chemotherapy and long remissions have been reported. Burkitt's lymphoma is of mature B cell derivation and expresses B cell surface antigen such as CD19, CD20, and CD22. Monoclonal surface IgM-κ or IgM-λ is also present and CD10 may be positive but TdT is weak or absent.

## Miscellaneous

### 1. Mycosis Fungoides and Se'sary Syndrome Cutaneous T-Cell Lymphoma

#### A. Mycosis Fungoides

Mycosis fungoides presents with inflammatory premycotic phase and progress through a plaque phase to a tumor phase.

Histologically: There is infiltration of the epidermis and upper dermis by neoplastic T cells, which have an extremely unusual cerebriform nucleus.

In most patients with progressive disease, extracutaneous manifestations, characterized by nodal and visceral dissemination appear.

#### B. Se'sary Syndrome

SS is related condition in which skin involvement is manifested clinically as a generalized exfoliative erythroderma. There is an associated leukemia of Se'sary cells that have the same cerebriform appearance noted in the tissue infiltrates of mycosis fungoides.

Fundamentally, both these disorders result from clonal proliferation of postthymic CD4 T lymphocyte.

#### C. Adult T-cell leukemia/lymphoma

This uncommon T-cell neoplasm has gained much prominence owing to its association with Human T-cell leukemia virus-I (HTLV-I).

Clinically: Skin lesion, lymphadenopathy, hepatosplenomegaly, Calcium increased, leukocytosis with multilobed lymphocytes. Neurological disorder is common. The tumor cells are CD4 (helper) and the lymphocytes express IL-2 receptor.

Both viruses can be transmitted by sex intercourse, blood products, contaminated needles, and from mother to her offspring.

**REAL Classification** A revised European-American classification of lymphoid neoplasms (REAL classification) has been proposed by the International Lymphoma Study Group (ILSG). This approach to lymphoma categorization attempts to define the diseases recognized with currently available morphologic, immunologic, and genetic techniques. This system incorporates new lymphoproliferative disorders that were not recognized by the Working Formulation and omits the general grading of lymphomas into low-, intermediate-, and high-grade categories.

The 13 most frequently occurring clinical entities that are recognized by the REAL classification are diffuse large B-cell lymphoma (31%), follicular lymphoma (22%), small lymphocytic lymphoma (6%), mantle cell lymphoma (6%), peripheral T-cell lymphoma (6%), lymphoma of mucosa associated tissue (MALT) type (5%), primary mediastinal large B-cell lymphoma (2%), anaplastic large (T-/null-cell lymphoma (2%), lymphoblastic lymphoma of T- or B-cell lineage, Burkitt's-like lymphoma (2%), marginal zone (monocytoid) B-cell lymphoma (< 1%), lymphoplasmacytic lymphoma (1%), and Burkitt's lymphoma (<1%). When immunotyping is used, the diagnostic accuracy of the REAL classification exceeds 85% for most subtypes, with the exception of Burkitt's-like and lymphoplasmacytic lymphomas, for which the classification has diagnostic accuracy rates of 53% and 56%, respectively.



**Table 15.2: The revised European American classification "REAL"**

B-cell Neoplasm	T-cell Neoplasm
Indolent Disseminated Lymphoma/Leukemia	
B-cell small lymphocytic /CLL/PLL Lymphoplasmatic lymphoma Hairy cell leukemia Plasmacytoma/myeloma Splenic marginal zone lymphoma	T-cell lymphocytic /CLL/PLL Large granular lymphocytic leukemia
Indolent Nodal Lymphomas	
Nodal marginal zone lymphoma +/- monocytoid B-cell lymphoma follicular center cell lymphoma (follicular lymphoma) grades 1,2,3 Mantle cell lymphoma	
Indolent Extranodal Lymphomas	
Extranodal marginal zone MALT +/- monocytoid B-cell lymphoma	Mycosis fungoids
Aggressive lymphoma	
Diffuse large	Anaplastic large cell Ki-1+ Peripheral T cell lymphoma Angioimmunoblastic Angiocentric Intestinal T
Highly Aggressive Lymphoma/Leukemia	
Precursor B-lymphoblastic leukemia/lymphoma Burkitt's lymphoma, Burkitt's like	Precursor T-lymphoblastic leukemia/lymphoma Adult T-cell lymphoma/leukemia (HTLV-1-ve)
Hodgkin's Disease	
Lymphocytic predominance +/- diffuse Classical HD: NS, MC, LD and lymphocyte-rich classical HD	
Anaplastic Large Cell Lymphoma, Hodgkin's Related	

### Diagnosis

No effective methods are available for screening or identifying populations at high risk for the development of NHL.

A definitive diagnosis can be made only by biopsy of pathologic lymph nodes or tumor tissue. A formal review by an expert hematopathologist for additional studies, such as immunophenotyping and genotyping, should be considered.

**Initial diagnostic evaluation** of patients with lymphoproliferative malignancy should include:

1. Careful history (night sweats, weight loss, fever; neurologic, musculoskeletal, or GI symptoms)
2. Physical examination (lymph nodes, including submental, infraclavicular, epitrochlear, iliac, femoral, and popliteal nodes; pericardial rub, pleural effusion, distended neck and/or upper extremity veins in superior vena cava syndrome; breast masses; hepatosplenomegaly, bowel obstruction, renal mass, and testicular or

ovarian mass; focal neurologic signs, such as plexopathy, spinal cord compression, nerve root infiltration, and meningeal involvement; skin lesions)

3. Biopsy of peripheral lymphadenopathy

4. Chest x-ray (mediastinal or hilar adenopathy, pleural effusions, parenchymal lesions)

5. CT scan of the chest (mediastinal, hilar, or parenchymal pulmonary disease)

6. CT scan of the abdomen and pelvis (enlarged lymph nodes, splenomegaly, filling defects in liver and spleen)

7. Bilateral bone marrow biopsy

8. Gallium scans (optional/selected cases)

9. Bone scan (selected cases) if musculoskeletal symptoms are present or alkaline phosphatase is elevated

10. CBC with differential and platelet count (peripheral blood lymphocytosis with circulating malignant cells is common in low-grade lymphomas). Bone marrow and peripheral blood involvement may be present, and the distinction between leukemia and lymphoma is difficult to make in some cases.

11. General chemistry panel,  $\beta_2$ -microglobulin are recommended

12. IV serology in patients with diffuse large cell, immunoblastic, and small noncleaved histologies; HTLV-1 serology in patients with cutaneous T-cell lymphoma, especially if they have hypercalcemia

13. Cytogenetic and molecular analyses of lymph node, bone marrow, and peripheral blood (selected cases)

14. Perform examination of CSF and strongly consider CNS prophylaxis in patients with (a) diffuse aggressive NHL with bone marrow, epidural, testicular, paranasal sinus, or nasopharyngeal involvement; (b) high-grade lymphoblastic lymphoma and small noncleaved cell lymphomas (Burkitt's and non-Burkitt's types); (c) HIV-related lymphoma; and (d) primary CNS lymphoma

15. Upper GI series with small bowel follow-through in patients with head and neck involvement (tonsil, base of tongue, nasopharynx) and those with a GI primary

16. Ultrasound of opposite testis in patients with a testicular primary

17. Spinal MRI scan for epidural disease when clinically indicated (useful in the evaluation of suspected spinal cord compression)

18. PET (FDG-glucose) scanning is gaining wider acceptance as a potential diagnostic approach for staging at diagnosis and relapse.

**PCR and Southern blot studies** Circulating monoclonal lymphoid cells can be detected by polymerase chain reaction (PCR) or Southern blot techniques, but the clinical utility of these studies is not well defined. Several studies have demonstrated the presence of circulating t(14;18)-positive cells in patients with durable remissions of follicular lymphoma, but whether this is a harbinger of relapse remains controversial. The t(14;18) translocation has been found in B-cells from blood of normal individuals, indicating that additional oncogenic events are necessary to establish the neoplastic phenotype.

## HODGKIN'S LYMPHOMA

Hodgkin's lymphoma is a malignant tumor closely related to other malignant lymphomas. It affects the lymphoid tissue primarily. It is characterized by progressive painless enlargement of lymphoid tissue through the body.

It is distinguished from NHL by the presence of Reed-Sternberg (RS) cells.

HL has an overall incidence in Yemen and has a bimodal age distribution with peaks of incidence in the third decade of life and over the age of 55 years. There is a slight excess incidence of HL in males.

### Etiology and Pathogenesis of HL

The origin of the malignant cell in Hodgkin's lymphoma is not firmly established.

RS cells and the associated abnormal and smaller mononuclear cells are neoplastic and that associated inflammatory cells represent a hypersensitivity response to the host. The RS cells regularly express major histocompatibility compound class II antigens and IgG fc receptors but are not phagocytic.

They express CD15, CD25 (IL2 receptor) and CD 30 and negative in most cases for CD 45 and for CD20. The reactivity with antibodies against CD30 is typical of Hodgkin's disease, but not specific, as activated lymphoid cells and anaplastic lymphomas are also positive.

Hodgkin's disease histogenetically is heterogenous from activated B cells and in others from T cells. EBV (Epstein Barr Virus) has been suspected as an etiologic agent on the basis of epidemiological and serological studies. The EBV genom has been detected in about 20-50% of cases in Hodgkin's tissue. It also has been found that Hodgkin's disease is frequent in individual with higher social status, less density of housing and smaller number of siblings. Recently, transforming growth factor  $\beta$ (TGF) has been characterized as the major immunosuppressive cytokine in Hodgkin's disease.

### Pathology

A distinctive neoplastic giant cell known as Reed-Sternberg (RS), is considered to be the essential neoplastic element in all forms of Hodgkin's disease, and its identification is essential for the histologic diagnosis. Classically it is a large cell (15-45um in diameter). Most often it is binucleate or bilobed with two halves often appearing as mirror images of each other. At other times there are multiple nuclei, or the single nucleus is multilobate and polypoid. The nucleus is enclosed within an abundant amphophilic cytoplasm. Prominent within the nuclei are large, inclusion-like "OWL-EYED" nucleoli generally surrounded by a clear halo. One variant of RS cells is called Lacunar cells is diagnosed and seen in nodular sclerosis. RS cells must be present in a bulk ground of non-neoplastic inflammatory cells, lymphocytes, plasma cells and eosinophils.



**Histological Classification:** (Rye Classification) Four subtypes of Hodgkin's disease can be distinguished according to histopathological criteria:

1. Lymphocyte predominance HD
2. Mixed cellularity HD
3. Lymphocyte depletion HD
4. Nodular sclerosis

**1. Lymphocyte Predominant Hodgkin's lymphoma (LPHL) :** LPHL is characterized by a diffuse or some times vaguely nodular infiltrate of mature lymphocyte admixed with variable numbers of benign histiocytes. Scattered among these cells are the distinctive RS cells. A majority of patients are males, usually under 35 years of age, and they present with limited disease. The prognosis is excellent. Without the identification of RS cells the lymphocyte predominance pattern could be readily mistaken for one of the lymphocytic form of NHL

**2. Mixed Cellularity Hodgkin's Lymphoma (MCHL):** MCHL is an intermediate clinical position between the lymphocyte predominance and the lymphocyte depletion. Typical RS cells are plentiful, but these are fewer lymphocytes than in lymphocyte predominance disease. The involvement of the lymph nodes is almost always diffuse.

**3. Lymphocyte Depletion Hodgkin's Lymphoma (LDHL):** LDHL is characterized by a paucity of lymphocytes and relative abundance of RS cells of their pleomorphic variants. It presents in two morphology forms, the so-called diffuse fibrosis and the reticular variants.

Diffuse fibrosis: The node is hypocellular and is replaced largely by a proteinaceous fibrillar material that represents a disorderly non-birefringent connective tissue. Pleomorphic histiocytes, a few typical and atypical RS cells and some lymphocytes.

Reticular variant: Is more cellular and is composed of highly anaplastic, large, pleomorphic cells that resemble RS cells.

**4. Nodular Sclerosis Hodgkin's Lymphoma (NSHL):** NSHL is characterized morphologically by two features including:

(a) The presence of a particular variant of the RS cells the Lacunar cells.

This cell is large and has a single hyperlobated nucleus with multiple small nucleoli and an abundant, pale staining cytoplasm. The phenotype of the lacunar cells resembles that of a B-lymphocyte (CD15-, CD20, 30, 45+).

(B) The other features seen in most cases are the collagen bands that divide the lymphoid tissue into circumscribed nodules.

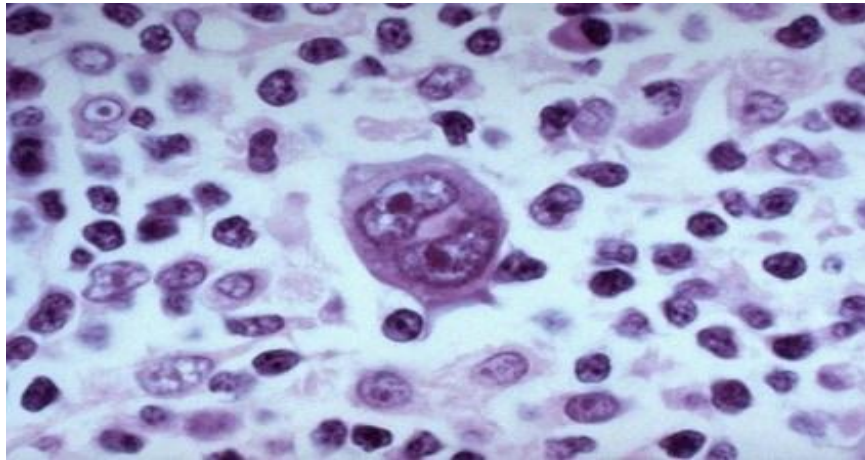
Classic RS cells are infrequent. Most of the patients are adolescents or young adults and they have an excellent prognosis especially when seen in clinical stages I and II.

The diagnosis of Hodgkin's disease rests solely on the unmistakable identification of the Reed-Sternberg cells in most variant and of the Lacunar cells in the nodular sclerosis pattern.

The REAL classification recognizes two main types of HL: classical types and nodular lymphocyte predominance type (NLPHL). The immunophenotype and genetic features of both classical HL and NLPHL have been defined.

The classical HL is defined by the presence of classic, diagnostic RS cells in a background of nodular sclerosis, mixed cellularity, lymphocyte rich, or

lymphocyte depletion, with the immunophenotype of classical HL (CD15+ CD30+, T and B associated antigens usually negative).



**Figure 15.1 : Hodgkins Lymphoma - Reed-Sternberg cells (RS)**

### **Clinical Features of Hodgkin's disease**

#### **1. Systemic Features**

Pel-Ebstein fever is a paroxysms of fever remaining for 10-14 days, followed by afebrile state for nearly equal period and so on, however, fever is commonly irregular. Lately there are anemia, anorexia, fatigue, weight loss, generalised pruritus and excessive sweating specially in cases complicated by secondary tuberculosis infection.

Alcohol ingestion may cause severe aching pain at sites of Hodgkin's involvement lasting for half to one hour.

2. Lymphnode enlargement is commonly the first manifestation and usually starts on one group and later involves other groups.

3. Abdominal manifestation includes splenomegaly in 50-60 %, liver enlargement and jaundice may occur in some cases. Ascites may follow peritoneal invasion or hepatic dysfunction. The enlargement of the retroperitoneal lymphnodes is common and may obstruct the ureters and inferior vena cava.

4. Chest manifestations in 50% of cases are dyspnea, cough, and stridor and chest pain due to enlargement of the mediastinal lymph nodes.

5. Neurological manifestation: Roots pain, herpes zoster and Horner's syndrome, cranial nerve palsy, spinal cord compression and peripheral neuropathy

6. Cutaneous manifestations: The common cutaneous manifestations are pruritus, hyperpigmentation, intracutaneous nodules, alopecia and exfoliative erythroderma.

7. Skeletal manifestations are bone pain and pathological fractures.



## Hematological Findings

Blood findings may vary from completely normal to markedly abnormal. Moderate normochromic normocytic anemia occurs, occasionally of the hemolytic type; may become severe. With marrow infiltrations BM failure may occur with a leukoerythroblastic anemia.

Peripheral blood changes in Hodgkin's disease are common (25% of cases at time of diagnosis) but not specific.

**Leukocytes:** may be normal, decreased or slightly or markedly increased (25000/ $\mu$ l). Leukopenia, marked leukocytosis, anemia are bad prognostic signs. Eosinophilia occurs in 20% of patients. If lymphocytosis is present, look for another disease. Neutrophilia and monocytosis may be found. These changes may all be absent or may even be present simultaneously or in various combinations. Rarely, Reed-Sternberg cells are found in marrow or peripheral blood smears in advanced disease.

Small lymphocytic and follicular lymphomas often have malignant lymphocytes in peripheral blood; in leukemic phase.

Cytopenia occur commonly due to hypersplenism, immune effect, or lymphoma effect on marrow.

ESR is increased and is useful for monitoring disease progress.

Bone marrow involvement at time of diagnosis in <10% of patients with Hodgkin's disease; 50% of patients with diffuse, small-cleaved lymphoma and mixed cell type: 70-80% of patients with follicular, small-cleaved cell lymphoma, less frequent in large cell lymphomas.

## Biochemical Findings

Increased serum LDH, in 30-40% of cases and indicates poor prognosis. Patients with bone disease may show hypercalcemia, hypophosphatemia and increased alkaline phosphatase.

Serum protein electrophoresis: Albumin is frequently decreased. Increased  $\alpha_1$  and  $\alpha_2$  globulins suggest disease activity. Decreased gamma globulins is less frequent in HD than in NHL. Gamma globulin may be increased, with macroglobulins present and evidence of autoimmune process (e.g., hemolytic anemia, cold agglutinins, and positive LE cells).

Elevated levels of transaminase may indicate liver involvement while increased serum bilirubin may be due to biliary obstruction caused by large lymph nodes

## Immunological Findings

Hodgkin's lymphoma patients commonly have deficiencies of cell-mediated immunity with increased susceptibility to bacterial, fungal, and viral (herpes zoster's and varicella) infections; these persist even after cure. Tuberculin test is usually negative due to loss of ability to show delayed hypersensitivity.

## Immunocytochemistry

Immunoenzymatic staining of RS cells on fresh lymphnodes has revealed staining with antigens specific for T cells (CD3, CD5 and CD2) or in some cases specific for B cells (CD19 and CD22).



Reed-Sternberg cells of Hodgkin's lymphoma are positive for CD15, CD25, the IL-2 receptor and CD30 and these considered as tumor marker for diagnosis.

### **Karyotyping**

It is clear that the involvement of specific chromosomes in numerical and structural abnormalities is non-random. Aneuploidy, or a deviation from the diploid number of chromosomes, resulting from the gain or loss of chromosomes or from polyploids, is a characteristic feature of Hodgkin's disease tumors that have an abnormal karyotype.

A gain of chromosome 1, 2, 5 and 21 is a recurring numerical abnormality; structural rearrangement involving chromosomal 1 is frequently observed.

### **Staging**

Accurate staging is extremely important. The natural history of the disease suggests that it arises in a single site and then spreads from one lymph (usually nodal) site to contiguous lymphoid sites and from lymphoid sites to contiguous nonlymphoid sites. It is difficult to prove that this is always the case, and in some cases it clearly is not but it is a useful concept that has led to a very successful strategy of staging and treatment. Thus, the primary objective of staging is to determine the current location of all the disease in the patient in order to plan a treatment that will address each of the involved area and all contiguous sites of possible spread

### **Clinical Stages of Hodgkin's and NHL**

The treatment depends on clinical stage and Ann Arbor classification is generally used:

Stage I: Single lymph node region or extralymphatic site. Example, nodes are on one side of the neck only.

Stage II: Two or more node regions on same side of diaphragm. Example, , nodes in the neck and chest.

Stage III: Lymph nodes on both sides of diaphragm; this may include spleen or localized extranodal site. Example, nodes are in the neck and retroperitoneum or the spleen.

Stage IV: Diffuse involvement of one or more extralymphatic organs. Example, nodes are in the chest and infiltration of the marrow and lung.

A: No constitutional symptoms

B: Presence of fever, night sweats/and/or weight loss over 6 months of 10% or more.

## Treatment of Lymphoma

**Table 15.3: International prognostic index (IPI) for NHL**

Risk factors			
1. Age > 60 years			
2. LDH > normal			
3. ECOG PS 2-4			
4. stage III-IV			
5. =2 extranodal sites			
Standard IPI score	Risk	CR%	OS at 5 y (%)
0-1	Low	87	73
2	Low- intermediate	67	51
3	High-intermediate	55	43
4-5	High	44	26

**Table 15.4: Follicular lymphoma International prognostic index (FLIPI)**

Risk factors			
1. Age > 60 years			
2. LDH > normal			
3. Stage III-IV			
4. >4 nodal areas			
5. =Hemoglobin <12 g/dl			
Risk	Factors	OS at 5 y (%)	OS at 10 y (%)
Low	0-1	91	71
Intermediate	2	78	51
High	=3	53	36

**Table 15.5: International prognostic score (IPS) for classical Hodgkins lymphoma**

IPS factors			
1. Serum albumin <4g/dl			
2. Hemoglobin < 10.5 g/dl			
3. Male gender			
4. Stage IV			
5. Age > 45 years			
6. WBC = 15000/ul			
7. Lymphocytes <8% or < 600/ul			
Risk factors	FPP at 5 y	OS at 5 y (%)	Patient %
0	84	89	7
1	77	90	22
2	67	81	29
3	60	78	23
4	51	61	12
>5	42	56	7

## Treatment of Hodgkin's Lymphoma

At present time the three treatment options for patients with newly diagnosed Hodgkin's disease are radiation, chemotherapy and the combination of both (combined modality)

Stage IA and IIA- Favorable: 4 cycles of ABVD followed by 3060 cGy IF XRT.

Stage IA and II A Unfavorable: 6 cycles of ABVD followed by 3060 cGy IF XRT.

Stage IB: 6 cycles of ABVD plus IF XRT if not in CR

Stage II B: 6 cycles of ABVD followed by 3060 cGy IF XRT.

Stage IIIA- IVB: 6-8 cycles of ABVD.

Consolidation Radiation: with 3060cGy will be given for areas of bulky disease on presentation except in stage IV.

### Treatment of recurrence:

Patients progressing on first line therapy or achieving less than CR (documented histologically) will receive HDC+PBSCT

Recurrence following radiation only: 6-8 cycles of ABVD

Recurrence following ABVD or MOPP/ABV:

- If less than 12 month DF interval: ESAP then PBSCT

- If more than 12 month DF interval:

- 6-8 cycles of MOPP/ABV or BEACOPP or

- ESHAP +/- HDC plus PBSCT or

- MOPP if initial chemo is ABVD

### Palliative chemotherapy regimens:

Novantrone, chlorambucil and prednisone

Single agent vinblastin

Single agent Etoposide.

### MOPP

Nitrogen Mustard	6 mg/m <sup>2</sup> iv	day 1+8
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Vincristine	1.4 mg/m <sup>2</sup> iv	day 1+8
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Procarbazine	100 mg/m <sup>2</sup> p.o	day 1-14
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Prednison	40 mg/m <sup>2</sup> p.o	day 1-14
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Repeat on day 29

### ABVD

Doxorubicin (Adriamycin)	25 mg/m <sup>2</sup> iv	day 1+15
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Bleomycin	10 mg/m <sup>2</sup> iv	day 1+15
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Vinblastin	6 mg/m <sup>2</sup> iv	day 1+15
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DTIC	375 mg/m <sup>2</sup> iv	day 1+15
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Repeat on day 29

### Other:

BEACOPP



## **Guideline treatment of Non-Hodgkin's Lymphoma**

**Bulky tumor:** any tumor diameter = 10 cm, Non Bulky tumor diameter < 10 cm.

### **Limited Stage**

Stage I or Stage II confined to 3 or fewer adjacent lymphnode regions with no B symptoms and non bulky.

### **Advance Stage**

Stage II with disease beyond 3 adjacent lymphnode regions or stage 3 or 4 or B symptoms or bulky tumor.

## **TREATMENT OF NHL INDOLENT LYMPHOMA**

### **Indolent, Stage I and Contiguous stage II NHL (Limited Stage)**

Involved field radiation, 3500-4000 cGY, the exact dose depending upon the bulk of the disease and its response during treatment.

In the circumstance when radiotherapy is contraindicated or not available chemotherapy can be applied for symptomatic patient or watchful waiting can be considered for asymptomatic patients.

Chemotherapy for Symptomatic patients: Single agent Chemotherapy: Chlorombucil.

Combination: CVP.

### **Non Contiguous stage II, III, IV (Advanced Stage)**

Observation for asymptomatic patients.

Oral alkylating agents (Chlorombucil, Cyclophosphamide orally). For nonresponder, progressive or relapsing patients other Choices include.

Combination Chemotherapy: CVP

Purine nucleoside analog: fludarabine

Fludarabine has now been shown to be more effective than CVP for both newly diagnosed or relapsed indolent lymphoma. It is reasonable alternative to CVP or chlorombucil, but we restrict its use to relapsed cases only due to availability and length of the treatment.

## **AGGRESSIVE NHL**

### **· Aggressive Stage I, and Contiguous stage II adult NHL: (Limited Stage) Non-bulky disease (<10cm)**

· CHOP X 3 and followed by radiotherapy, 4000 - 5000 cGY; the daily dose from 180 - 200 cGY. The radiotherapy ports include all visible sites of disease determined before biopsy and treatment with CHOP.

Patient who obtained CR with chemotherapy will be treated with IFXRT (3600 cGY/4w/20fr) and those who achieved less than CR will be treated with IFXRT (4000-5000 cGY/4-5 w/20-25 fr).

· CHOP X 6-8 for those who refuse radiotherapy after 3 cycles.

**Bulky disease (=10cm)**

CHOP X 6-8 (2 cycles after documentation of CR) plus involved field radiotherapy, 3600 – 5000 cGY.

CR: IFXRT (3600 cGY/4w/20fr)

Less than CR @ IFXRT 4000-5000 cGY/4-5 W/20-25 fr)

Patients with progressive disease (PD) should be considered for radiation therapy or to be involved in study protocol or treated according to discretion of his physician.

**Aggressive Non Contiguous stage II, III, IV NHL (Advance Stage)**

·CHOP X 6 – 8

·Patients with high risk for relapse (IPI 3-5) are candidate for Consolidation with high dose chemo and peripheral stem cell transplant and should be considered for that when resources become available.

· **CNS prophylaxis with Intrathecal methotrexate 6 Injections is recommend for patients with:**

·Para Nasal Sinus Lymphoma

·Testicular lymphoma

·Lymphoblastic and Burkitt's Lymphoma

**Relapsed NHL**

· **Indolent lymphoma**

· Observation for asymptomatic patient

· Single agent chemotherapy or Combination chemotherapy CVP, CHOP

· Involved field radiation

· Fludarabine

· Rituximab

**Aggressive, Relapsed NHL**

HDCT, PBSCT for chemo sensitive, < 60 years with no bone marrow or CNS involvement, after 2 cycles of salvage chemotherapy e.g. ESAP

**Other Choice**

· Enroll in Study Protocol or

· ESAP X 6 and in case of progression, or no response to ESAP or unable IMVP-16 X 6, preferably treatment given as out patients.

**Management for Special sites of lymphoma****Eye Lymphoma**

· *Orbital/Peri orbital soft tissue lymphoma* – Radiation using a lens sparing technique.

Chemotherapy with agents suitable for low-grade lymphoma can induced prolonged remission and it is the best choice if disease is wide spread at diagnosis or recurs after radiation.

· *Intra ocular lymphoma and Optic nerve lymphoma*– irradiation to include both eyes using a lens sparing technique (if possible), with corticosteroid ±. HD MTX. In case of relapse treatment include HD MTX or Ara-C.

- **Conjunctival Lymphoma** – usually of low grade and should be treated with irradiation.

#### **Primary CNS lymphoma**

The diagnosis should be based on biopsy of the lesion if possible. All patients should have HIV antibody testing.

- Old Patients > 60 ys, patients with poor performance status, with (non HIV) PCNS lymphoma: Whole brain irradiation to 4,000 cGY in 20 fractions, followed by a “boost” to the tumor site to 5,000-5,400 cGY in 6-8 fraction + Dexamethasone till end of irradiation or improvement of symptoms
- Young patients or Old patients with good performance status: Chemo (HDMTX with leucovorin rescue or HD Arac) for 3 cycle followed by Irradiation to whole brain as above.

#### **Lymphoma of the Para nasal Sinuses**

##### **Phased treatment approach:**

- Systematic treatment
- Localized disease - CHOP X 3
- Advanced, B symptoms, Bulky: CHOP X 6-8 cycle
- Local Rx: Irradiation at least 3,600 CGY. In 20 fractions if the patient is in CR, otherwise boost to a higher dose, for all patients treated with short chemo or any advanced stage with residual disease. After a full course of chemotherapy.

#### **T-cell/NK cell paranasal**

**lymphoma – should have radiotherapy upfront followed by chemotherapy.**

- CNS Prophylactic; Intrathecal Methotrexate 12 mg six doses over 3 weeks.

#### **Testicular Lymphoma**

- Usually DL B-cell Lymphoma and usually aggressive even at early stage.

##### **Phased Management approach**

Diagnosis should be established by orchidectomy, and standard staging tests for lymphoma.

Stage I AE, I BE – CHOP X 6-8 + Whole scrotum Radiation (entire scrotal contents). 3,600 cGY in 20 fraction if in CR. Otherwise add boost.

Stage 2 AE, 2BE – CHOP X 6-8 + Whole scrotum Radiation + IFNRT.

Stage III, IV, A or B symptoms, Bulky or Non Bulky: CHOP X 6-8 + radiation of the whole scrotum and remaining testis as above.

For Stage III, IV: Intrathecal chemotherapy 6 doses over 3 weeks. At the end of planned chemo treatment.

#### **Gastric Lymphoma**

All patients with GI lymphoma should have a careful ENT Examination.

##### **Indolent Lymphoma (Other than MALT)**

- Early stage Irradiation
- Advance stage as appropriate



.. **Diffuse large cell lymphoma of Stomach - (DLCL) - B-cell type**

- Stage I AE or II AE: Could be treated with CHOPX3 + upper abdominal irradiation or CHOPX6 for those Pt. Who refused irradiation.
- Stage III, IV, B symptoms, bulky disease (> 10 cm): CHOP 6-8 cycles with irradiation reserved for any residual disease after chemo completed.
- Resection of gastric lymphoma is no longer recommended and reserved for cases with perforation or bleeding only.

**Old Patients with aggressive lymphoma (= 65 years).**

The optimal approach to an older patient with aggressive lymphoma may be similar to that of young patients e.g. CHOP, unless there is contra indication of usage of anthracycline. CNOP is less preferable. Dose reduction not indicated from first cycle especially if patient has good performance status and normal hepatic profile and now data indicating a superior survival for elderly patients when Rituximab used with chemotherapy, and this policy will be adapted when the drug become available.

For elderly patients with poor performance status and those expected not tolerate conventional induction therapy a number of modified regimens have been developed for their use, usually there regimens incorporate reduced dosage of known active drugs for weekly therapy

**Post transplantation lympho proliferative disorder (PTLD)**

Occurs in about 1-3% of recipients of organs transplant almost always associated with Epstein Barr virus.

**Treatment according to types**

.. Polyclonal forms – withdrawal of immuno suppressive drug if not successful or not feasible Combination acyclovir +  $\mu$ -Interferon. If failed – CHOP X 6 for disseminated disease and local RT for localized presentation. **Rituximab** (Anti CD 20) is effective immunotherapy for these patients and should be considered if progression reoccur after reduction of immunosuppressive drugs and before using combination chemotherapy.

**Monoclonal forms**

Multifocal and rapidly aggressive

- Modification of immuno suppressive
- Interferon
- And Combination chemotherapy CHOP X 6.

For failure after chemotherapy immunotoxin (anti CD 20 B cell surface antigen antibody).

**AIDS related lymphoma**

- **HIV infection + CD4 count lymphocytes < 200**
- Low dose: CHOP (50%)  $\pm$  GCSF
- **HIV Patients with CD4 counts > 200**
- Full dose: CHOP  $\pm$  GCSF

- **Primary CNS lymphoma in AID Patients**
- **Radiation to brain + Decadron.**

**COP (CVP)**

Cyclophosphamide 400 mg/m<sup>2</sup> iv or oral day 1-5  
 Vincristine 1.4 mg/m<sup>2</sup> iv day 1  
 Prednison 100 mg/m<sup>2</sup> p.o or iv day 1-5  
 Continue on day 22

**CHOP / R-CHOP**

Cyclophosphamide 750mg/m<sup>2</sup> iv day 1  
 Adriamycin 50mg/m<sup>2</sup> iv day 1  
 Vincristine 1.4mg/m<sup>2</sup> iv day 1  
 Prednisolon 60mg p.o day 1-5  
 Rituximab 375 mg/m<sup>2</sup> iv day 1

Continue on day 22.

Drug dosages are depends from WBC and platelet count/ $\mu$ l on the day of administration.

Courses are indicated every 3 weeks for 6-8 courses.

**CHOP**

WBC	Platlets	Dose
>4000	>130000	100% all drugs
3900-2500	129-80000	50% CPA+ADR, 100% VCR
2500-1500	7200-50000	50% VCR only
<1500	<50000	No drugs, except prednisone

R-CHOP: Rituximab-CHOP

F-R: Fludarabine-rituximab

**NHL- High Grade****Centroblastic Lymphomas**

Stage II/II<sub>E</sub> -----Radiotherapy + Chemotherapy

Stage III-IV -----Chemotherapy (CHOP)

For recidive ----- (COP-BLAM)

**COP-BLAM**

C: Cyclophosphamid 400 mg/m<sup>2</sup> iv day 1  
 O: Vincristin 1 mg/m<sup>2</sup> iv day 1  
 P: Prednison 40 mg/m<sup>2</sup> p.o day 1-10  
 BL: Bleomycin 15 mg iv day 14  
 A: Adriamycin 40 mg/m<sup>2</sup> iv day 1  
 M: Procarbazin 100 mg/m<sup>2</sup> p.o day 1-10

Continue on day 22 ---- for 6 cycles.

If there is no response then use IMEP for only 2 courses.

**Immunoblastic Lymphomas**

Stage I/I<sub>E</sub>-II -----Radiotherapy then Chemotherapy (CHOP)

Stage III-IV -----Chemotherapy (COP-BLAM)(6 cycles)

Alternative (CHOEP) (4 cycles)

### CHOEP

Cyclophosphamid	750 mg/m <sup>2</sup>	iv	day 1
Adriamycin	50 mg/m <sup>2</sup>	iv	day 1
Vincristin	2 mg/m <sup>2</sup>	iv	day 1
Etoposid	100 mg/m <sup>2</sup>	iv	day 1-3
Prednison	100 mg	p.o	day 1-5

Relapse after CHOP:

1. local relapse -----> RADIOTHERAPY
2. Systemic relapse -----> CHEMOTHERAPY (IMEP)

### IMEP

IFOSFAMID	1000 mg/m <sup>2</sup>	iv	day 1,2,3,4,5
METHOTRXAT	30 mg/m <sup>2</sup>	iv	day 3 and 10
ETOPOSID	100 mg/m <sup>2</sup>	iv inf.	day 1-3
PREDNISON	40 mg/m <sup>2</sup>	p.o	day 1-5

CONTINUE ON DAY 22

### Burkitt's lymphoma

Staging:

A: single extraabdominal tumor

B: multiple extraabdominal tumors

C: intraabdominal tumor including kidneys or gonads

D: intraabdominal tumor with one or more extraabdominal manifestation (BM, CNS)

AR: Stage C but 90% of the tumor operated

Prognosis is improved when bulky abdominal tumor can be resected. Then chemotherapy with:

### COMP

Cyclophosphamide	1000 mg/m <sup>2</sup>	iv	day 1
Vincristine	1.4 mg/m <sup>2</sup>	iv	day 1
Methotrexate	12.5 mg/m <sup>2</sup>	i.th	day 2+5
Methotrexate	12.5 mg/m <sup>2</sup>	iv	day 1-5
Prednison	100 mg/m <sup>2</sup>	iv	day 1-5

Repeat the cycle on day 15-22

### MALT-Lymphomas

Like other low-grade lymphomas, MALT lymphomas are probably incurable with standard chemotherapy approaches. The treatment approach to MALT lymphomas is similar to that used for other low-grade NHLs. MALT lymphoma affecting the stomach is associated with previous *H pylori* infection, but a causative role for this organism is unproven. Complete eradication of *H pylori* after treatment with bismuth and/or omeprazole (Prilosec), amoxicillin, and metronidazole frequently leads to disappearance of symptoms and histologic complete remission. In most cases, some evidence of histologic response to treatment of the *H pylori* infection is evident 2-3 months following eradication of the organism.



**H Pylori associated MALT:-** Multiagent antibiotic, follow-up endoscopy, if H. Pylori not eradicated second course of antibiotic is indicated. MALT may take long time to regress.

**Persistence MALT:-** Six months after eradication: Single agent chemotherapy e.g.: Chlorambucil or upper abdominal radiation. Patient with H. Pylori and MALT lymphoma need long term follow up these patients may relapsed years or decades later

**Eradication therapy for H. Pylori in MALT lymphoma**

- Omeprazole 20 mg PO BID
- Clarithromycin 500 mg PO BID
- Amoxicillin 1gm PO BID or Metronidazole 500 mg PO BID
- Duration 2 weeks.

Surgical and chemotherapy intervention

In low-grade malignant lymphomas (stage I-II) after surgical intervention, the radiotherapy is indicated. Chemotherapy is according to the condition of the patient.

**Radiotherapy**

It is indicated in the following cases:

1. Lymphoma of Waldeyer's ring
2. Thyroid gland lymphoma
3. Mycosis fungoides
4. Primary CNS lymphoma.

**REVIEW QUESTIONS**

1. When Reed-Sternberg cells are found in a lymph node biopsy, they are indicative of
  - a. Hodgkin's disease
  - b. Intermediate grade
  - c. Sézary syndrome
  - d. High Grade lymphoma
2. The Reed-Sternberg cell is characterized as having
  - a. A thin nuclear membrane
  - b. Only one nucleus
  - c. Large nucleoli with a distinct halo
  - d. A smaller size than normal lymphocyte.
3. The definitive diagnostic test for Hodgkin's disease is a
  - a. Complete blood count
  - b. Serum iron quantitation
  - c. Bone marrow biopsy
  - d. Lymph node biopsy
4. Lymph node biopsies in NHL show mostly
  - a. Many normal cells mixed with a small number of neoplastic cells
  - b. Many uniform, similar appearing neoplastic cells
  - c. Many Reed-Sternberg cells
  - d. Malignant T lymphocytes

5. During treatment for lymphoma, one common effect of a single course of combination chemotherapy includes a significant.

- a. Decrease in granulocytes
- b. Increase in platelets
- c. Decrease in erythrocytes
- d. Increase in leukocytes

6. Mycosis Fungoides and se'zary syndrome are rare lymphomas characterized by:

- a. Neoplastic B cell migration to the skin
- b. Small peripheral blood lymphocyte with small, clefted or folded nuclei
- c. Large peripheral blood lymphocytes with cerebriform nuclei.
- d. Large peripheral blood lymphocytes with normal appearing nuclei.

7. Which of the following features is not characteristic of the Reed-Sternberg cell?

- a. Similar in size to a megakaryocyte
- b. Bilobate or multilobate nuclei
- c. "OWL-eyes" appearance
- d. Strongly positive PAS staining

8. Which of the following drugs is not included in the MOPP chemotherapy drug program?

- a. Mustargen
- b. Vincristine
- c. Methotrexate
- d. Procarbazine
- e. Prednisone

9. Which of the following drugs are included in the CHOP chemotherapy program?

- a. Doxorubicin
- b. Vinblastin
- c. Procarbazine
- d. Hydroxyurea

10. Which of the following characterizes NHL of childhood?

- a. Generally nodular in histology
- b. Usually indolent rather than aggressive
- c. Often lymphoblastic, presenting as a mediastinal mass
- d. Almost never with T-Cell surface markers.

11. The following statements are related to Non-Hodgkin's lymphoma except

- a. Are more likely to be T cell than B cell lineage
- b. Occur more frequently in patients with HIV infection
- c. Are more likely to be disseminated (stage IV) when the histology is of indolent disease than when histology shows aggressive disease..
- d. Are more common than Hodgkin's lymphoma

12. The following factors play a role in the development of NHL except:

- a. Pesticides used for khat, vegetables and fruits
- b. Shigellosis and salmonellosis
- c. EBV and HTLV-1

- 13: QUIZ:** What is the diagnosis
- a. Plasmacytoma
  - b. Sézary syndrome
  - c. B-cell lymphoma
  - d. Dermatitis





Multiple myeloma is a B lymphoid malignancy, which is characterized by the proliferation of a malignant clone of plasma cells, which synthesize and secrete excessive amounts of monoclonal immunoglobulin. MM is a disease of the elderly, with the median age at diagnosis being about 62 years.

**Pathophysiology**

The pathological and clinical features of myeloma are due to:

1. Tissue infiltration
2. Production of large amount of paraprotein
3. Impairment of immunity.

Bone infiltration, causes destruction of medullary and cortical bone due to the stimulation of osteoclast activity by a factor released by the myeloma cells, usually designated osteoclast-activating factor. This leads to osteoporosis and more frequently to localized lytic lesions and pathological features.

Skeletal destruction results in the release of bone salts, negative calcium balance and hypercalciuria in virtually all cases.

Hypercalciuria → osmotic diuresis + Impairment of renal tubular reabsorption

→ Dehydration → diminished urine output → hypocalcemia + azotemia → anorexia, nausea, and vomiting and further dehydration.

Production of large amount of paraprotein results in a wide variety of abnormalities as, raised serum globulin level, hypoalbuminemia, hyponatremia, dilutional anemia, raised ESR, rouleaux in blood film, hyperviscosity, interference with platelets function and coagulation pathway.

Proteinuria, renal failure, amyloidosis occurs in 10% of patients, cryoglobulinemia in 5% of patients'

**Clinical Feature**

1. Pain is most frequent in the tubular, sacral and thoracic spine, and in the rib cage, but it also occurs in the hips, legs, shoulders and arms (Rheumatic pain). It is uncommon in the skull. Tenderness of the bones is common and the problems are basically due to pathological feature, lysis or compressions.
2. Symptoms of hypercalcemia.
3. Symptoms of anemia, which includes pallor, general weakness, dyspnea, and tachycardia.
4. Infections: Are common in myeloma, is the cause of morbidity and mortality. Chest infection with streptococcus pneumoniae. The cause is subnormal levels of normal Ig and suppression of the normal antibody response, in particular the primary response.

5. Renal insufficiency: May occurs in upto 30% of patients and is caused by hypercalcemia, infection, deposition of paraprotein or light chain, uric acid or amyloid.
6. Neurological symptoms: A variety of neurological complications may be troublesome in MM. The most serious such complication, compression of the spinal cord by localised tumor deposits in the vertebrae or epidural space, is manifest as sensory loss, incontinence and paraplegia.
7. Bleeding disorder
8. Symptoms of hyperviscosity; the common symptoms are lassitude, confusion, coma, blindness, bleeding, infection, renal failure, hypertension and CCF.

#### **Blood Picture**

1. Increased of ESR (up to 100% due to increase serum globulin)
2. Anemia: Often present at diagnosis or it develops during the course of the disease  
Usually normochromic normocytic

#### **Causes of anemia in MM**

- A dilutional effect of the expanded plasma volume in patients with high concentrations of paraprotein contributes to the lowering of the hemoglobin concentration.
  - Depression of erythropoiesis is due to infiltration of the bone marrow, and the effects of cytotoxic chemotherapy.
  - Renal failure, chronic infection and bleeding.
  - Developing of myelodysplastic or leukemic disorders.
3. Leukocytes: May be normal, raised, or moderately reduced, but moderate leukopenia is common, especially in advanced disease and in association with cytotoxic therapy.  
A leuko-erythroblastic picture with immature red cells and granulocytes develops in 10% of patients. 40-55% lymphocytosis is frequently with variable immature lymphocytes. In some cases small number of myeloma cells appear in 20% of patients and rarely in large numbers in plasma cell leukemia. Eosinophil may be increased
  4. Platelets count is often reduced in myeloma.

#### **Blood Film**

The stained film shows short rouleaux formation of 2-4 cells. (Rouleaux formation is causing difficult blood cross matching due to changes of protein. Bluish ring appears around each cell due to a proteinaceous.

#### **Bone Marrow**

Plasma cells are >10% (10-50% or more) and present of abnormal forms of myeloma cells, such as less clumping of nuclear chromatin and present of large nucleoli, lack of a perinuclear zone and lighter blue cytoplasm. Flow cytometry of the aspirate can be used to quantitate the malignant plasma.

## Blood Chemistry

**Protein:** Total serum protein concentration is often increased due to presence of paraprotein ranged from 70-120/l but may be higher and associated with reduction in albumin concentration. (with decrease A/G ratio in 50-75%).

**Immunological Deficiency:** Increase susceptibility to bacterial infection, particularly pneumococcal pneumonia, this generally reflected in a decrease in serum concentration of normal IgA, IgG, IgM and serum protein electrophoresis reveals abnormal protein in 80% of patients.

60% of patients with IgG myeloma protein (complicated by hypercalcemia and heavy Bence Jones proteinuria is usual. 20% of patients with IgA myeloma protein. 1-10% of patients without abnormal protein. 1% of patients are with IgD myeloma protein (commoner in younger patients and hypercalcemia and renal failure are frequent).

Bence Jones proteinuria occurs in 50-60% of patients. More recently, it has been found that Bence Jones proteinuria is occasionally associated with specific defects in renal tubular reabsorption mechanism. Bence Jones protein is a low M.W, heat sensitive urinary protein found in multiple myeloma, which coagulates when heated at 50°C and redissolves particularly or wholly on boiling (100°C).

## Biochemical Serum Finding

Serum calcium is increased.

Kidney diseases of multiple myeloma:

BUN and uric acid are increased. Renal function test is decreased

Urine abnormalities with presence albumin cast.

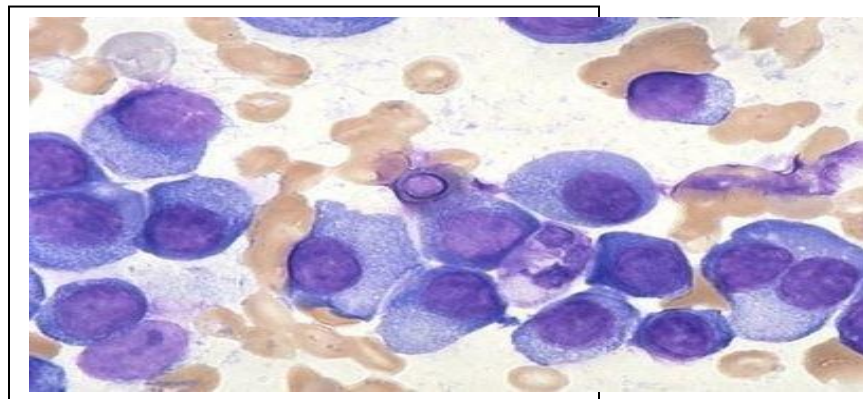
Renal failure is usually present when there is a marked increase of Bence Jones protein in the blood.

Hemorrhage diathesis in 10% of patient due to:

Some normal clotting factors are absorbed by the abnormal plasma protein.

A pathological effect on platelets by the paraprotein often lead to abnormal platelet function, with prolongation of the bleeding time and defective adhesion and aggregation.

Increased fibrinolytic activity is coupled with thrombocytopenia



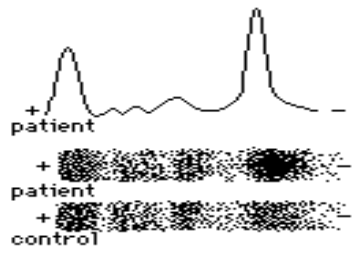
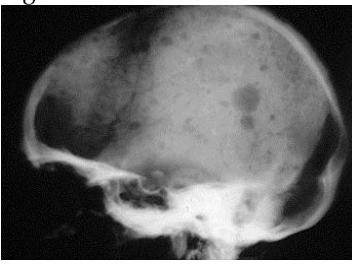
**Figure 16.1: B.M smear : Multiple myeloma (Plasma cells )**

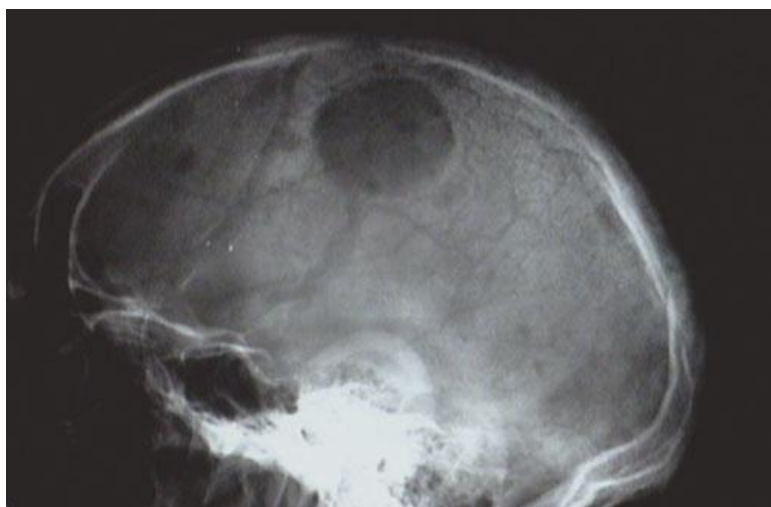


**Table 16.1: Diagnostic criteria for multiple myeloma**

<b>Major Criteria</b>	
Marrow plasmacytosis (>15%) with expression of a single heavy and light chain class of immunoglobulin	
Serum M-component >3.5g/dl of IgG or > 2g/dl of IgA	
Urinary light chain excretion >1 g/24h of a single class ( $\kappa$ or $\lambda$ )	
A biopsy proven plasmcytoma.	
<b>Minor Criteria</b>	
Less than 15% plasma cell in the marrow but with predominance of one immunoglobulin light chain class.	
An M-component quantitatively less than specified above.	
Depressed level of normal (non M-component) immunoglobulin	
Unexplained normochromic, normocytic anemia	
Serum $\beta$ 2-microglobulin level of >4 mg/litre	
Unexplained renal dysfunction	
Unexplained hypercalcemia	
The diagnosis of myeloma requires at least one major and one minor criterion or at least three minor criteria including A and B.	

**Table 16.2 : Stages of plasmacytoma according to DURIE AND SALMON 1975)**

Stage	Criteria	Plasma cells (cells $10 \times 10^9/m^2$ )	
I	All of the following 1.Hb >9 g/dl 2.Serum calcium normal 3.one isolated osteolysis 4.paraprotein concentration IgG <5g/dl, IgA <3g/dl Bence Jones proteinuria <4g/24h	<0.6	 <p>Serum protein electrophoresis shows the appearance of a monoclonal band in the g region</p>
II	Criteria between I and III		
III	One of the following 1.Hb <8 g/dl 2.Serum calcium >12 mg/dl 3. Bone lesion 4. IgG >7g/dl, IgA >5g/dl Bence Jones proteinuria >12 g/12h	>1.2	 <p>Osteolytic lesions of skull</p>
<b>A: Normal renal function (creatinine &lt; 2mg/dl)</b> <b>B: Abnormal renal function (creatinine &gt; 2mg/dl)</b>			



**Figure 16.2 :** The rounded "punched out" lesions of multiple myeloma appear as lucent areas of this skull radiograph

**Table 16.3: International staging system criteria**

• Stage	• International Staging System Criteria
• I	• $\beta_2$ -microglobulin < 3.5; albumin $\geq$ 3.5
• II	• Neither stage I nor stage III values
• III	• $\beta_2$ -microglobulin > 5.5

### Treatment

The current trend is to wait and see or to treat with supportive care only stage II and I.

#### ● **Goals of treatment**

Address pain relief & other disease symptoms

Control disease activity

prevent further organ damage

Extend disease-free survival (DFS)

Prolong overall survival (OS)

Preserve normal performance and QOL for as long as possible

Chemotherapy should be adopted only in those stage III patients with one or more of the following features: Hb<8.5g/dl Serum  $\text{Ca}^{++}$  >12mg/dl, >3 lytic bone lesions, High M-protein production rate (IgG >70g/l or IgA>50g/l

Surgical intervention (local therapy) is indicated in patients with isolated plasmocytom.

Stage I: The proper policy of treatment is waiting and watch.

Stage II: Chemotherapy.

- **First-Line:**
  - VAD
  - MP
  - Transplant (depending on age)
  - **Second Line:**
  - VAD
  - Dexamethasone
  - Thalidomide
  - Transplant
  - Investigational Therapy
  - **Refractory:**
  - Supportive or palliative care
  - Investigational Therapy
- Deaths 12,000/yr

#### **Novel therapies**

Bortezomib (Velcade)

Thalidomide and analogues

Trisenox (Arsenic Trioxide)

Genasense (bcl-2 antisense)

Farnesyl Transferase Inhibitors

VMP (Velcade+Melphalan+Prednisolone) significantly prolongs survival in the largest MP-based phase III study

Consistency of treatment effect .

Rapid and durable responses with very high Complete Response rate (similar to transplantation).

Prolonged time to progression

#### **MP**

Melphalan 0.25 mg/kg p.o day 1-4

Prednison 2 mg/kg p.o day 1-4

1 mg/kg p.o day 5

Continue every 3 weeks according to CBC

For alternative or failure management with melphalan we give:

Cyclophosphamid 1g/m<sup>2</sup> iv day 1

Prednison 100-200 mg p.o day 1-4

Continue program of chemotherapy every 3 weeks

Addition of Mesna (urometixan), 200mg/m<sup>2</sup> IV used to protect mucous membrane.

Thalidomide + Dexamethasone

Thalidomide 200 mg/d p.o continuously

Dexamethasone 40 mg/d d1-4, d9-12, 17-20 every 4 weeks



Bortezomib +/- dexamethasone

Bortezomib 1.3mg/m<sup>2</sup> iv bolus d1,4,8,11 every 3 weeks for 8 cycles.

#### **Supportive**

Biphosphonates- zoledronic acid or pamidronate for preventing skeletal-related events in patients with MM

Erythropoietin

Prophylactic antibiotics especially TMP-SMX

Plasmapheresis

Low-dose radiation therapy can be used for palliation of uncontrolled pain, impending pathologic fracture, or spinal cord compression

#### **Stem cell or bone marrow transplantation**

High-dose chemotherapy followed by autologous stem cell transplantation is now considered the treatment of choice for multiple myeloma patients with intermediate or high stages of disease.

### **WALDENSTROM: PRIMARY MACROGLOBULINEMIA**

It is a malignant lymphocyte-plasma cell proliferative disorder with abnormally large amount of gamma globulin of the IgM type. The basic abnormality in this macroglobulinemia is uncontrolled proliferation of lymphocyte plasma cells.

It is most commonly found in older men; the mean age of onset is about 60 years. The onset is usually insidious.

#### **Clinical Features**

The common manifestations are weakness, fatigue and bleeding.

1/4 of patients are with neurological abnormalities.

The incidence of infection is twice the normal rate. Chronic anemia, thrombocytopenia and hyperviscosity contribute to the bleeding.

#### **Laboratory Findings**

The lymphocyte-plasma cells vary morphologically ranging from small lymphocytes to obvious plasma cells. This cytoplasm is frequently ragged and may contain periodic acid schiff (PAS) positive.

Leukocytes are normal with an absolute lymphocytosis. Red blood cells show Rouleaux formation due to hyperviscosity. There is moderate to severe degrees of anemia. ESR is increased. Abnormalities in platelet adhesion and factor VIII may be low.

Serum electrophoresis: Overproduction of IgM (IgM) antibodies; cryoglobulin may be detected.

Cryoglobulin are protein that precipitate or gel when cooled to 0°C and dissolved when heated. In most cases, monoclonal cryoglobulin's are IgM or IgG.

#### **Treatment**

WM behaves clinically as low-grade lymphoma and, generally the treatment is palliative and conservative. Symptomatic relief from

viscosity syndrome is achieved most readily by repeated plasmapheresis. Progressive disease is treated with alkylating agents such as chlorambucil, melphalan and cyclophosphamide.

### QUESTIONS REVIEW

1. At the time of diagnosis, the peripheral blood film of a patient with multiple myeloma typically shows:
  - A. Greater than 10% plasma cells
  - b. Marked lymphocytosis
  - c. Neutrophilia
  - d. Rouleaux formation
2. A patient is suspected to have multiple myeloma. Serum protein electrophoresis appears to be normal. The laboratory should
  - a. Examine the urine for Ig light chain
  - b. Repeat the serum protein electrophoresis
  - c. Measure plasma viscosity
  - d. Perform an erythrocyte sedimentation rate.
3. A patient's bone marrow is found to have increased numbers of plasma cells, plasmacytoid lymphocytes, and mast cells. The patient's serum most likely has an M composed of:
  - a. IgA
  - b. IgM
  - c. IgG
  - d. Heavy chain, only
4. Which of the following manifestations of multiple myeloma would be LEAST likely to be seen on chest X-ray features?
  - a. Lytic rib lesions associated with rib fracture
  - b. Multiple lytic lesions of vertebral bodies
  - c. Vertebral rarification and collapse
  - d. Plasmacytoma producing coin lesion in lung periphery
5. The abnormal protein frequently found in the urine of persons with multiple myeloma is:
  - a. Albumin
  - b. IgM
  - c. IgG
  - d. Bence Jones
6. Waldenstrom's macroglobulin is characterized by increased level of:
  - a. IgG
  - b. IgM
  - c. IgD
  - d. IgA
7. Multiple myeloma is a disorder of:
  - a. T-lymphocyte
  - b. Plasma cells
  - c. Megakaryocytes
  - d. The lymph nodes

Injury to the vessel wall exposes collagen and sets it in motion, a series of events leading to hemostasis.

Hemostasis is a complex process, depending on interactions between the vessels wall, platelets, coagulation factors and fibrinolytic system. If a blood vessel endothelium is injured, 4 events take place at the same time.

1. Initially, rapid vasoconstriction reduces blood flow and promotes contact activation of platelets and coagulation factors and this produce:

Prostacycline that inhibits platelet aggregation in non- injured area, and factor VII.

Endothelial damage leads to:

Release of tissue thromboplastin, which activates extrinsic pathway (factor VII).

Exposure of subendothelial collagen, which lead to:

Platelet adhesion and aggregation

Activation of factor XII (Extrinsic pathway).

Activation of fibrinolysis.

2. In the second phase, platelet adheres immediately to the exposed subendothelial connective tissue, particularly collagen. The platelets are aggregates to form platelet plug.

Release of ADP, which increases further platelet release reaction serotonin and local vasoconstriction by releasing thromboxane A<sub>2</sub> production and vasoconstriction and increase platelet release reaction.

Production of phospholipid (PF<sub>3</sub>), which has a local antiheparin action, so it enhances local clotting.

Finally, platelet thrombasthenin lead to formation of the firm hemostatic plug.

3. In the third phase, activation of intrinsic or extrinsic clotting systems to produce fibrin clot. Depression of fibrin stabilizes platelet plug and which lead to firm hemostatic plug.

4. Finally fibrinolysis occurs following the release of tissue plasminogen activators from the vascular wall. Fibrinolytic removal of excess hemostatic material is necessary to re-establish vascular integrity.

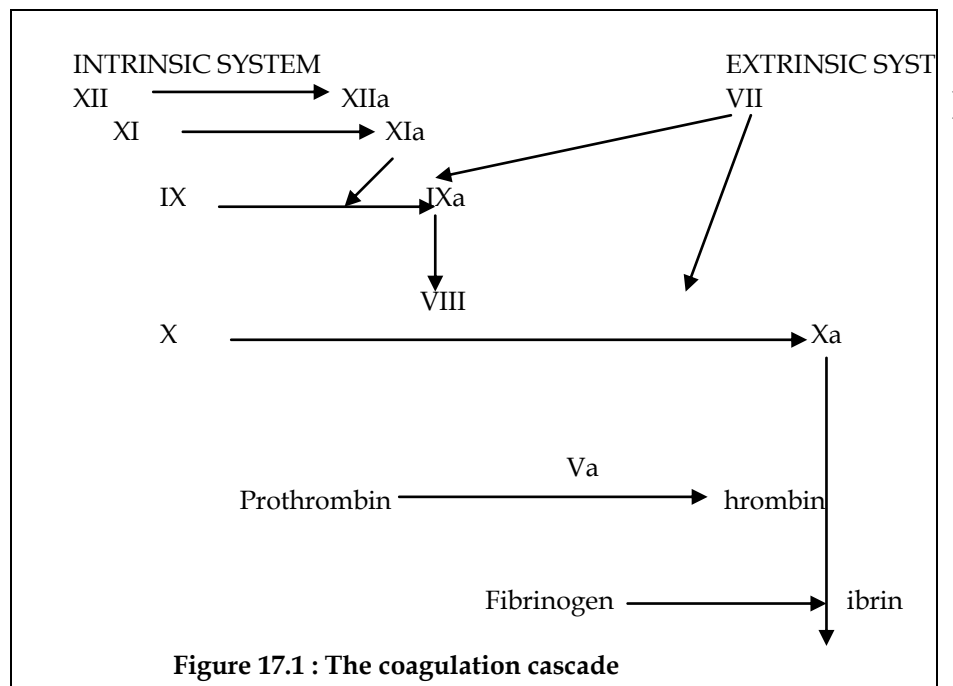
### Causes of Hemorrhagic Diseases

1. Vascular abnormalities
2. Quantitative and qualitative platelet deficiency
3. Defective coagulation mechanism



**Table 17.1 Nomenclatures of coagulation factors**

Factors	Name	Synthesis	Function
I	Fibrinogen	Hepatocyte	Substrate
II	Prothrombin	Hepatocyte/ vitamin K	Enzyme
III	Tissue thromboplastin	EC+ other cells	Receptor/cofactor
IV	Ionized Calcium	Hepatocyte/EC/Platelets	Cofactor
V	Proaccelerin	Hepatocyte/	Cofactor
VII	Proconvertin	Hepatocyte/ vitamin K	Enzyme
VIII	Antihemophilic globulin	Sinosoides of liver	Cofactor
IX	Christmas factor	Hepatocyte/vitamin K	Enzyme
X	Staurt-Prower factor	Hepatocyte/vitamin K	Enzyme
XI	Plasma thromboplastin antecedent	Hepatocyte	Enzyme
XII	Hagman factor	Hepatocyte	Enzyme
XIII	Fibrin stabilizing factor	Hepatocyte/platelets	Transglutaminase
--	Prekalikerin Fletcher	Hepatocyte	Enzyme
--	High molecular weight Fitzgerald factor.	Hepatocyte	Cofactor



**Figure 17.1 : The coagulation cascade**

### CLOTTING REACTION

The first reaction in the sequence is the cleavage of factor X by factor VIIIa and tissue factor to form factor Xa. This activity is greatly enhanced during the clotting sequence by cleavage factor VII to VIIa (1)

$VII+X \rightarrow Xa$  (weak)

$Xa+VII \rightarrow VIIa$

$VIIa+X \rightarrow Xa$  (strong reaction)

The Second reaction is the cleavage of factor IX to IXa. This is mediated by factor VIIa with tissue factor as helper: (2) VII (TF) + IX  $\rightarrow$  IXa

The third reaction is the cleavage of factor X to Xa by factor IXa and its helper, factor VIIIa. Reaction (2) and (3) are critical to sustaining the clotting sequence, since the tissue factor-factor VIIa complex is inhibited quickly

(3) IXa (VIIIa) + X  $\rightarrow$  Xa

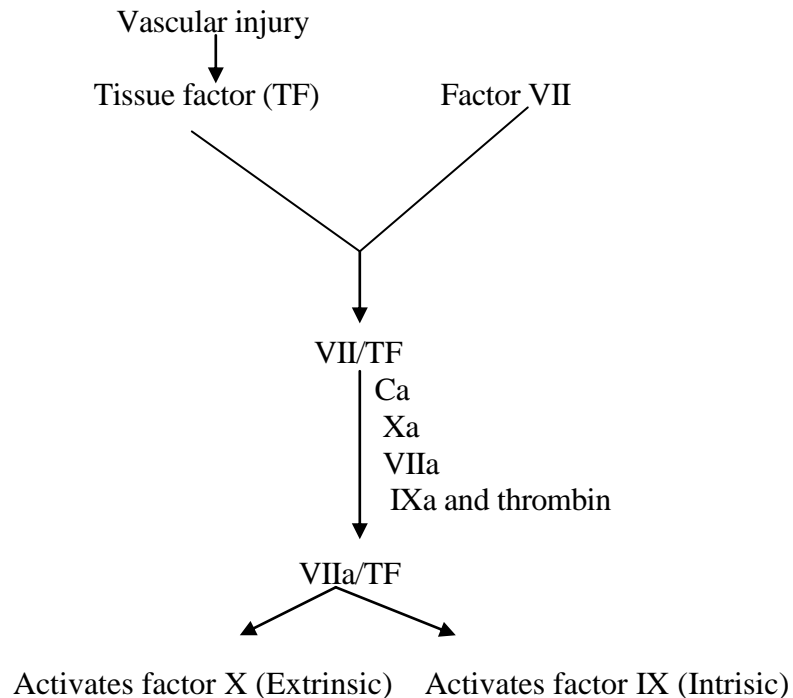
Xa + VIII  $\rightarrow$  VIIIa (feedback loop)

The fourth reaction is the conversion of factor II (prothrombin) to IIa (thrombin) by factor Xa and its helper Va. Minute amounts of thrombin activates V and VIII to Va and VIIIa

(4) Xa (Va) + II  $\rightarrow$  IIa

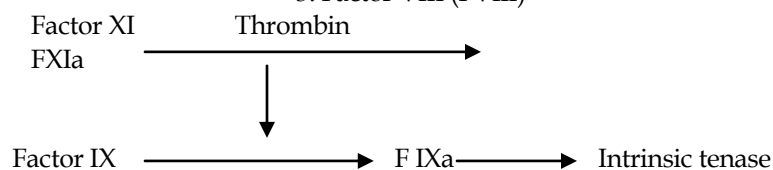
**Figure 17.2 Extrinsic pathways (Tissue factor pathway)**

- Main component  
1. Tissue factor (TF)  
2. Factor VII (FVII)

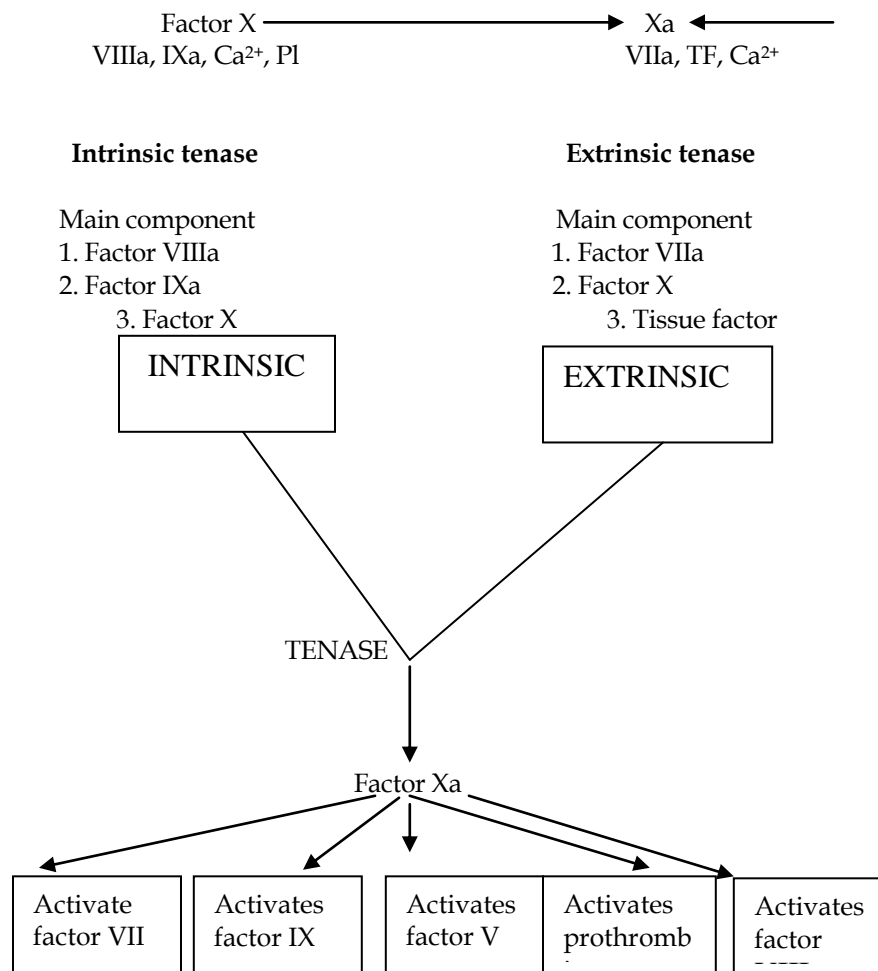


**Figure 17.3 Intrinsic pathway**

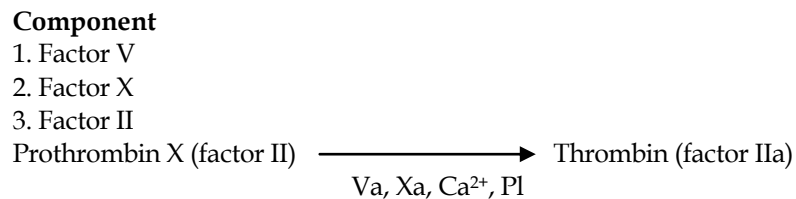
- Main component  
1. Factor XI (FXI)  
2. Factor IX (FIX)  
3. Factor VIII (FVIII)



**Figure 17.4 Common pathway**



**Figure 17.5: Prothrombinase complex**  
**GENERATION OF THROMBIN**

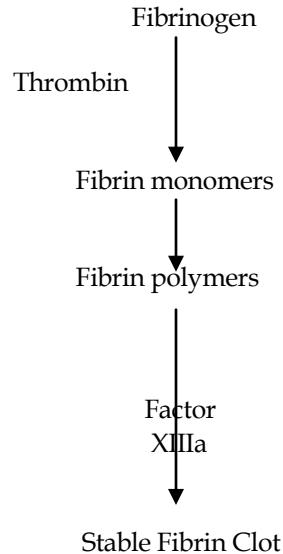




**Figure 17.6 : Fibrin clot formation**

Main components

1. Thrombin
2. Fibrinogen
3. Factor XIII

**Contact System**

This system includes the following factors:

1. XII
2. XI
3. Prekallikrein
4. High molecular weight kininogen

Upon vascular and exposure of subendothelial basement membrane or collagen, these factors interact together and with the exposed area, leading to:

1. Activation of factor XII with subsequent activation of clotting system.
2. Activation of prekallikrein, which leads to:
  - Further activation of factor XII
  - Activation of plasminogen to plasmin → fibrinolysis
  - Possible activation of complement pathway.

Except for factor XI deficiency that leads to hemophilic 'C' of either of these factors cause no bleeding tendency. Diagnosis is by finding a prolonged PTT with normal PT, in absence of bleeding tendency. Correction of the test with the deficient factor confirms the specific diagnosis.

**Inhibitors of Coagulation**

Removal of activated factors by rapid blood flow at the periphery limits coagulation to the site of injury.

Anti-thrombin III (AT-III) is the most potent inhibitors of the coagulation. It is a heparin cofactor. It forms inactive stable complexes with activated factors XIIa, XIa, IXa, Xa, thrombin, Kallikrein, Plasmin. The presence of this protein is the basis for use of heparin as anticoagulant.

Protein C is activated by thrombin into active protein C, which destroys factors V and VIII in the presence of another protein called protein S. It also leads to activation of fibrinolysis.

Other natural inhibitors of coagulation are:

Alpha1-antitrypsin, alpha1-antiplasmin and alpha2-macroglobulin

The fibrinolytic system: When activated, it leads to degradation of fibrin to FDPs.

### Fibrinolysis

Inactivated plasminogen is activated by plasminogen activators into plasmin.

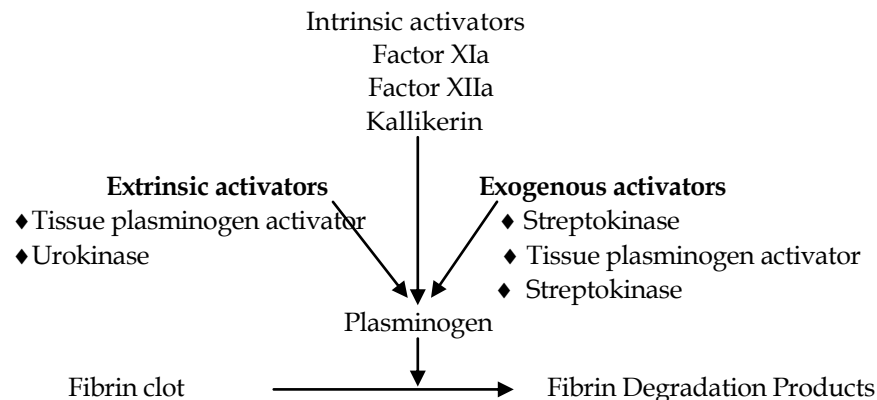
Plasmin acts on both fibrinogen and fibrin, leading to their fragmentation into degradation products (FDPs or fibrin and fibrinogen degradation products).

FDPs are normally rapidly removed from circulation by liver and RES.

Plasminogen activators and inhibitors control the system.

### Activators

1. Tissue type plasminogen activators produced by vascular endothelium.
2. Urokinase, produced by kidney.
3. Kallikrein, produced from pre-kallikrein under the effect of active factor XIIa.
4. Thrombin, produced during coagulation.



### Inhibitors

1. Alpha-antitrypsin
2. Alpha1-antiplasmin
3. Alpha2-macroglobulin
4. Anti-thrombin III

### Signs and Symptoms Suggests Systemic Hemostatic Failure

The characteristics of hemorrhage may point towards the presence of systemic hemostatic failure including:

1. Inappropriate local hemorrhage
2. Bleeding from multiple sites.
3. Unexplained bruising
4. Purpura, ecchymosis, buccal or retinal hemorrhage
5. Oozing from wounds or venipuncture sites.

### **Features Suggests of Platelet Disorders**

Mucocutaneous hemorrhages of Skin, nose, gums, vagina, and gastrointestinal tract.

The most common signs are purpura and cutaneous ecchymosis.

Any bleeding appears intraoperatively or immediately postoperatively.

Tourniquet test is positive.

### **Features Suggests Coagulation Disorders**

Deep hemorrhage of muscles and joints

Palpable ecchymoses

Delayed of posttraumatic and postoperative hemorrhage.

### **The Medical Conditions Which Maybe Associated With Hemostatic Feature**

There are several conditions, which maybe complicated by hemostatic features, including:

1. Primary hematological disorders.
2. Conditions associated with DIC.
3. Liver diseases
4. Uremia
5. Anticoagulant therapy
6. Antiplatelet therapy
7. Endocrine disorder
8. Vitamin K or C deficiency
9. Malabsorption syndrome
10. Massive blood transfusion.

### **Laboratory Investigations**

#### **(A) Test for Evaluation of Platelet Role in Hemostasis**

##### **1. Platelet Count**

- The normal platelet is 150000 -400000/ $\mu$ L
- Spontaneous bleeding occurs if count is < 40000/ $\mu$ L 'Thrombocytopenic purpura'.

##### **2. Hess Test (Tourniquet Test; Capillary Fragility Test)**

A blood pressure cuff is inflated around arm for 15 minutes, between systolic and diastolic pressure. Normally less than 5 petechiae appear in an area on the forearm 2.5 cm square. An increased number indicates of thrombocytopenia or platelet dysfunction or abnormalities of small blood vessels.

##### **3. Ivy Bleeding Time**

A blood pressure cuff is applied to the arm, and inflated to 40 mmHg.

A stab incision, 2mm long and deep is made using a scalpel blade.

At 30 seconds interval, blood is blotted from its margin till bleeding stops.

It is prolonged in abnormal vascular and platelet phases of hemostasis.

N.B: when the platelet count is under 40000/ $\mu$ L, bleeding time and tourniquet test give abnormal results.



#### **4. Platelet Function Test**

Fresh citrated platelet-rich plasma is used for platelet aggregation test. A platelet aggregometer is used to examine the response of platelets to aggregating agents, including collagen, adrenalin, ADP, arachidonic and ristocetin; from these studies it is possible to examine the primary and secondary aggregation responses, the release reaction and abnormalities suggesting Von Willebrand's disease.

5. Bone marrow smears for number and morphology of megakaryocytes.

6. Peripheral blood smears for morphology of platelets (shape and size)

#### **(B) Tests for Evaluation of the Clotting System**

##### **1. Whole Blood Clotting Time**

A crude test for the entire coagulation mechanism.

It is the interval for a firm blood clot to form in a glass test tube.

##### **2. Thrombin Time**

This is a test of the final conversion of fibrinogen to fibrin and bypasses the intrinsic and extrinsic systems as thrombin is added to the test system.

Diluted thrombin is added to citrated plasma in a concentration, which will clot normal plasma in 10-15 seconds. A prolongation of the time is caused by:

Deficient substrate-hypofibrinogenemia

Defective substrate-dysfibrinogenemia

Inhibitors-antithrombin action of heparin, inhibition of fibrin polymerization due to FDPs, high levels of protease inhibitors in the acute phase reaction (e.g alpha-2-macroglobulin).

##### **3. Prothrombin Time (PT)**

The PT is a test of the extrinsic pathway where citrated plasma is recalcified at the same time as tissue factor (thromboplastin) is added the clotting time recorded.

PT is used to assess the extrinsic VII and common Pathway (X, V, II, I).

The time needed for plasma to clot after addition of thromboplastin and  $\text{Ca}^{++}$ .

If phase II is normal, prolonged PT indicates deficiency of factors II, V, VII, or X.

This test is used to monitor oral anticoagulant therapy (Double the normal time).

##### **4. Partial Thromboplastin Time (PTT).**

It needs normal intrinsic (XII, XI, IX, VIII) and common (X, V, II) pathways.

The time required for plasma to clot after activation by kaolin,  $\text{Ca}^{++}$ , brain extract (platelet substitute).

It is used to assess factors XII, XI, IX, VIII (phase I), when TT and PT are normal.

**5. International Normalized Ratio (INR)**

Differences in commercially available thromboplastin, (tissue factor preparations) result in different sensitivities to the deficiencies of coagulation factors. Thus, on the basis of a patient PT and the normal PT, the INR is calculated:  $INR = (PT \text{ of patient} / PT, \text{ mean normal})$

The use of INR permits doctors to obtain the appropriate level of anticoagulation independent of laboratory reagents and to follow published recommendations for intensity of anticoagulation (Normal INR is 1).

**6. Specific Factor Assay:** for the clotting factors by immunoassay.

**7. Euglobulin Lysis Time:** Is shortened if low factor XIII and in fibrinolytic states.

**8. Clot Solubility** in 5M-urea solution: Is rapid in low factor XIII

**9. Plasma FDPs:** Are increased in case of DIC and fibrinolytic states.  
(For more details Appendix 1)

**Table 17.2: Interpretation and follow up of screening test**

TT	PTT	PT	Platelet	Bleeding T	Problem and further investigations
Normal	Normal	Increase	Normal	Normal	?Ext. Path Look for factor VII ? Liver ? Warfarin
Normal	Increase	Normal	Normal	Normal	? Int. Path F VIII+XI Lupus
Normal	Increase	Normal	Normal	Increase.	? von Willebrand
Normal	Increase	Increase	Normal	Normal	Defect of Appropriate Common pathway Factors Liver/warfarin
Increase	Increase	Increase	Normal	Normal	Fibrinogen/or heparin
Normal	Normal	Normal	Increase	Increase	Thrombocytopenia /B Marrow
Normal	Normal	Normal	Normal	Increase	Abnormal. Platelet Function

REMEMBER: When TT is normal:

1. If PT is prolonged + normal PTT → defect of factor VII
2. If PT is normal + prolonged PTT → defect of XII, XI, IX, and VIII
3. If both PT and PTT are prolonged → defect of X, V, or II



## COAGULATION DISORDERS

### A.Phase I Disorders:

1. Hemophilias (deficiency of factors VIII, IX, or XI) and Hagman factor deficiency.
2. Von Willebrand disease.
3. Any of these leads to prolonged PTT, and a normal TT and PT.

## HEMOPHILIA A (Classical Hemophilia)

Hemophilia A is a sex linked disorder due to a deficiency of factor VIII. It accounts for 85% of the hemophilia and has a population incidence of 20 per 100,000 males. The clinical severity may vary from severe disease (factor VIII <1%) with spontaneous hemorrhage 2-4 times a month, to mild disease factor VIII C >5% when hemorrhage usually only occur in relation to trauma.

### Etiology

HA is an inherited X-Linked-recessive disease, characterized by deficiency of factor VIII clotting activity (VIIIc).

### Clinical Picture

HA characterized by excessive bleeding induced by minor trauma or injury. Hemarthrosis, which is repeated, especially affecting the knees, ultimately fibrous ankylosis with limitation of movement occurs. Bleeding is common after circumcision, or tooth extraction (prolonged and severe). Spontaneous epistaxis and hematuria may occur. Subcutaneous ecchymoses and hematomata after IM injection. Severe internal or intracranial hemorrhage may occur with severe trauma.

Table 17.3: Laboratory characteristics and clinical manifestations

Laboratory defects (%)	Clinical manifestation	Bleeding symptoms
<1	Severe	Early childhood, spontaneous
1-5	Moderate	After minor trauma or may be spontaneous
5-20	Mild	Only after trauma or surgery

Bleeding time is normal bleeder's disease. Clotting time is prolonged.

Abnormal phase I test: Prolonged partial thromboplastin time (PTT) and prothrombin consumption time. These can be corrected by plasma adsorbed to Ba SO<sub>4</sub> but not by serum.

Plasma VIIIc is low or absent, while VIII Ag is normal.

**Diagnosis of Female Carriers:** By low factors VIIIc and factor VIII c Ag (50% of normal), and with normal f VIII R Ag.

**Prenatal diagnosis** is used in male fetus of carrier females. Fetal blood taken by fetoscopy (at 20-22 weeks) is assayed for VIIIc and VIII Ag as in adults.

Amniotic fluid fibroblasts show DNA polymorphism in case of hemophilia.

Trophoblastic biopsy can be used for diagnosis as early as 10-12 weeks gestation (using DNA polymorphism).

### **Treatment**

Acute bleeding episodes are treated with concentration of clotting factor VIII in hemophilia A and clotting factor IX in hemophilia B. The recombinant factor VIII concentrates have been induced in the treatment regimens mainly of previously untreated persons. Spontaneous bleeding (joints, muscles), is usually controlled if the patient's factor level is raised to 20% of the normal level. If the hemorrhage is occurring at critical sites (CNS, nasopharyngeal area), before major surgery or after serious posttraumatic bleeding, the factor VIII or IX level should be elevated to 100% and then maintained above 50% until healing has occurred. The amount of clotting factor needed can be calculated as follows: 1 unit of factor VIII/kg will raise the blood level by 1%; and 1 unit of factor IX/kg will raise the blood level by 1-2%.

DDAVP provides an alternative treatment for increasing the factor VIII levels in patients with a mild form of hemophilia A.

A residual factor VIII activity (usually >10%) is required to obtain a moderate rise in the patient's factor VIII level following the intravenous administration of 0.3 µg/kg DDAVP.

The patient should be advised to avoid aspirin, other antiplatelet drugs and intramuscular injections and should be registered with a recognised hemophilia centers and should carry a card with details of their conditions.

### **HEMOPHILIA B**

HB is a factor IX deficiency "christmas disease". It is an X-linked recessive. It is about 15% of all hemophiliacs and differs from Hemophilia A in:

HB is less common and less severe clinical picture. There is less frequent hemarthrosis.

Prolonged PTT can be corrected by normal serum, not by Ba So adsorbed normal plasma.

### **HEMOPHILIA C**

HC is a factor XI deficiency. It is an autosomal recessive inheritance. Both males and females are affected. It is less common (1% of all hemophilias) and less severe than hemophilia A.

Prolonged PPT can be corrected by either BaSo<sub>4</sub> adsorbed normal plasma or by serum.

## VON WILLEBRAND DISEASE

It is an autosomal dominant disorder characterized by a prolonged bleeding time associated with deficiency, or a qualitative defect in von willebrand factor function.

### Clinical Picture

It is characterized by abnormal bleeding; usually starting in childhood. It affects both males and females. Family history of bleeding tendencies is common.

### Laboratory Finding

Prolongation of the bleeding time is a hallmark in the diagnosis of von Willebrand disease due to reduced platelet adhesion and vascular abnormality.

Clotting time and partial thromboplastin time (PTT) are prolonged; Factor VIIIc and VII-Ag are reduced. Prothrombin time is normal.

Defective platelet aggregation to ristocetin is found in some patients due to absence of VW-factor. Mild thrombocytopenia may occur.

### Types of Von Willebrands Disease

1. Type I classical (A-C): There is available decrease of F VIIIc, factor VIIIIR;Ag and VIIIIR;Co factor to 10% to 40% of normal.
2. Type II (A-H): There is a specific absence of the high molecular weight multimers of VIIIIR: vWf in both the plasma and platelet (type IIa) or solely in the plasma (type IIb)
3. Type III: Severe form of the disease; also called severe type I by some.

### Treatment

Most patients with moderate vWD (usually type I) and mild bleeding symptoms or bleeding after minor surgery with respond to DDAVP, which is given in a dosage of 0.3µg/kg intravenously over 30 minutes. DDAVP probably releases the very large vWF multimers from the endothelial cells or platelets and thus corrects the prolonged bleeding time. DDAVP should not be given in types 2B and type 3vWD. In these subtypes, bleeding episodes must be treated with intermediate-purity factor VIII concentrates that contain vWF and factor VIII.

## B.PHASE II DISORDERS

Factor II, V, VII, X (prothrombin complex).

Deficiency of any of these factors leads to prolonged prothrombin time (PT).



N.B: All these factors are synthesized in liver vitamin K dependent factors are II, VII, IX, X. Factor V is formed in the liver but is not vitamin K dependent.

### **Malabsorption of Vitamin K**

This may result from:

Biliary obstruction or atresia i.e lack of bile salt (vitamin K is fat soluble).

Malabsorption syndromes

GIT sterilization (an alteration of bacterial flora), which is responsible for production of vitamin K.

### **Liver Disease**

Liver disease leads to defects of coagulation, platelets and fibrinolysis.

1. Impaired synthesis of vitamin K dependent factors (II, VII, IX, X).
2. Impaired synthesis of factor V and fibrinogen (severe hepatic damage).
3. Thrombocytopenia due to hypersplenism (cirrhosis).
4. Increased fibrinolysis.
5. Reduced levels of protein C and S, antithrombin and  $\alpha_2$  antiplasmin lead to susceptibility to DIC.

### **C-PHASE III DISORDERS**

All have a prolonged thrombin time (TT)

1. Congenital Afibrinogenemia
2. Congenital dysfibrinogenemia
3. Factor XIII deficiency (fibrin stabilizing factor)

### **Platelet**

Platelets are Non-nucleated small cellular fragments. They are produced by megakaryocytes in bone marrow, by budding. Life span in circulation is 7-10 days.

Normal platelet count is 150000-400000/ $\mu$ L. Bleeding disorders usually associates platelet counts below 40000/ $\mu$ L.

Platelet functions are intimately involved in both vascular and clotting mechanisms:

1. Adhesion
2. Aggregation
3. Release of: ADP, serotonin, TX-A<sub>2</sub>  
PF<sub>3</sub> (essential for clotting)  
PF<sub>4</sub>- antiheparin
4. Contraction: platelets are necessary for clot retraction
5. Adsorption of clotting factors to their surfaces.

Activation of platelets occurs in response to:

Collagen contact: exposed by vascular injury

ADP

Serotonin

Thromboxane A<sub>2</sub>

Thrombin: produced from coagulation system

## PURPURA

In purpura, there are:

Petechiae; is a minute extravasations of blood from very small blood vessels.

Ecchymoses are more extensive hemorrhages of purpura.

Bleeding is from mucus membrane and into other organs and tissues.

### Classification

#### I. Non-thrombocytopenic purpura

(a). Defects in small blood vessels

- Allergy vasculitis-Henoch-Schonlein purpura
- SLE
- Infections- meningococcal septicemia, measles, typhoid
- Vitamin C deficiency, dysproteinemias
- Connective tissue disorders e.g Eher-Donlos.

(b) Defective platelet function

- Drug induced: Aspirin, Dipyridamole
- Thrombasthenia (Glazmann's)
- Bernard-Solier
- Storage pool disease

#### II. Thrombocytopenic purpura

(a) Excessive destruction or utilization

- Immune; ITP
- Hypersplenism
- DIC, Thrombotic thrombocytopenic purpura Hemolytic uremic syndrome.
- Mechanical -prosthetic heart valve.
- Drugs, Infections.

(b) Inadequate production

- Aplasia of bone marrow- Aplastic anemia.
- Inhibition of bone marrow: Irradiation, Cytotoxic drugs.
- Bone marrow replacement, acute and chronic leukemia, lymphoma, and myelofibrosis.
- Megaloblastic anemia
- Drugs
- Infections, measles, dengue fever, EBV
- PNH

## IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

ITP is an autoimmune disorder where autoantibodies (IgG) are directed against the platelets, which are subsequently destroyed by the monocyte macrophage system predominant in the spleen.

### Clinical Features

The onset is either acute or insidious. Two forms of idiopathic thrombocytopenic purpura are recognised: acute and chronic:

#### (A). Acute Purpura Hemorrhage

1. Occurs most frequently in children and young adults and is somewhat commoner in females than in males.
2. Onset is abrupt, commonly following viral infections. Bleeding is spontaneous in the form of petechial hemorrhages, bruises or echymosis but hematoma is very rare.
3. Bleeding from orifices as epistaxis, bleeding from the gums, hemoptysis, hematemesis, hematuria, melena and uterine bleeding.
4. Internal bleeding is a serious and may be fatal as hemoperitoneum, hemopericardium and subarachnoid hemorrhage.
5. Spleen is rarely palpable and platelet count is very low.
6. Spontaneous remission usually occurs in 10 days to 3 months.

#### (B). Chronic Thrombocytopenic Purpura

1. Usually in premenopausal women, gradual onset with remissions and exacerbations.
2. Hemorrhage may be spontaneous or traumatic.
3. The spleen is uncommonly palpable; indeed, splenomegaly is a point against the diagnosis of ITP.
4. Capillary fragility test is positive.

### Laboratory Investigations

Decreased number of blood platelets, hemorrhagic occurs when platelet count is  $<40000/\mu\text{L}$ . Also abnormalities in platelet size and morphology are common.

Prolonged bleeding time and Hess test is positive. Clotting time is normal. Clot retraction is poor.

**Bone Marrow:** Megakaryocytes are increased in number but not surrounded by much platelet they are abnormal with single nuclei and vacuolated scanty cytoplasm.



## Treatment

The first issue is to decide if a patient requires any specific treatment or not. Patients with moderate thrombocytopenia (platelets  $> 40000/\mu\text{l}$ ) with no history or signs of bleeding do not require therapy and should just be observed. In comparison, patients with clinical signs of bleeding or very low platelet counts usually  $<20000/\mu\text{l}$ , require therapy. Initial therapy consists of corticosteroids prednisone or prednisolone 1-2 mg/kg/d, which usually will raise the platelet count to safe level in 70-60% of the patients within 1-2 weeks. When the platelet count has risen above  $100000/\mu\text{l}$ , one can begin to taper the dose of corticosteroids down to 10-15 mg/day. Only a few patients (10-20%) will have longstanding remissions following treatment with corticosteroids. Unfortunately, the platelet count of most adults with ITP will fall as the dose of corticosteroids is reduced. Splenectomy is indicated in patients who become unresponsive to corticosteroids or require prohibitively high dose to maintain the platelet count at an acceptable level. In patients with an initial response to splenectomy followed by a relapse, the possibility of an accessory spleen should be ruled out. Because subsequent treatment poses increased risk to the patient it should be decided if treatment in failing patients is absolutely necessary.

Treatment options in such patients include "pulse dose" dexamethasone, (40 mg/d 1-4 every 4 weeks), azathioprine, cyclophosphamide, vinca alkaloid, and danazole.

High doses of IV IgG (0.4 g/kg day 1-3 for 3-5 days or 1 g/kg day 1 for 1-2 days) raise the platelet count in about 60-70% of ITP patients. For patients with life threatening bleeding, or preoperatively if corticosteroids have failed, IgG is the treatment of choice.

## DISSEMINATED INTRAVASCULAR COAGULOPATHY (DIC)

DIC is an acquired thrombohemorrhagic disorder that results from the effects of excessive formation of thrombin and plasmin in circulation. DIC is always secondary to an underlying pathologic process that triggers activation of blood and generation of thrombin.

The causes of DIC are:

1. Intravascular hemolysis
2. Infection
  - a. Septicemia purpura fulminans
  - b. Postsplenectomy sepsis, meningococcemia (Gram negative)
3. Obstetric complications
4. Burns, electric shock and 1. Shock (from any cause)
5. Heat stroke
6. Fat embolism
7. Surgery- cardiac bypass surgery, lung, brain
8. Envenomation
9. Malignancies- AML, prostatic, GIT
10. Pancreatitis
11. LeVeen shunts
12. Vascular disorders: hemangiomas, vasculitis

DIC is characterized by the consumption of clotting factors and platelets within the circulation, resulting in varying degrees of microvascular obstruction.

When significant platelet and coagulation factor consumption occurs, bleeding becomes a major feature.

A secondary fibrinolysis occurs, which in some cases, may accentuate the bleeding:

These events may lead to:

1. Tissue ischemia and necrosis
2. Generalized hemorrhagic state
3. Hemolytic anemia

A wide spread activation of the coagulation system, with consumption of clotting factors occurs with the following results:

1. Reduction of the circulating clotting factors and platelets "consumption"
2. Blood becomes incoagulable, and severe bleeding manifestations may occur.
3. Thrombosis in the small blood vessels, which leads to tissue ischemia, infarction and Necrosis.
4. Hemolytic anemia of the microangiopathic type, with prominent fragmented RBCs "Schistocytes in the blood smear".

### **Clinical Features**

Some patients are asymptomatic with only laboratory evidence of DIC.

Symptoms and signs due to DIC:

- a). Hemorrhagic manifestations which vary in severity.
- (b). Tissue thrombosis may involve many organs and infarctions of large areas of skin and subcutaneous tissue, or of kidneys.
- (c). Microangiopathic hemolytic anemia; due to red cell distortion and fragmentation in small blood vessels.
- (d). Renal and adrenal failure may occur, due to intravascular thrombosis.

### **Laboratory Finding**

Prolonged TT, PT, PTT

Hypofibrinogenemia, thrombocytopenia and low factor VIII

Fibrin split products (FSP) appear in the blood, due to activation of fibrinolysis.

Blood smear show fragmented RBCs, schistocytes. These changes are referred

as microangiopathic hemolytic anemia.

### **Treatment**

1. Treat the cause, e.g antibiotic, removal of the procoagulant stimulus (e.g a dead fetus).
2. Supportive therapy with fresh frozen plasma, platelet concentrates and cryoprecipitate if bleeding is dominant
3. Anticoagulant therapy (e.g heparin) if thrombosis is dominant.
4. Protein C and antithrombin in selected patients.

## Thrombosis

Thrombosis may occur in either the arterial or venous circulation. It is the pathological process whereby platelets and fibrin interact with vessel wall to form a hemostatic plug to cause vascular obstruction (Virchow's triad).

### Virchow's Triad

1. Stasis
2. Hypercoagulable state
3. Endothelial plug

### Mechanisms

- A. Endothelial damage
- B. Blood flow
  1. Stasis
  2. Turbulence
  3. Hyperviscosity
- C. Blood components
  1. Platelets
  2. Thrombin
  3. Factor VIII
  4. Fibrin (dysfibrinogenemia)
  5. Plasminogen activator deficiency
- D. Hypercoagulability
  1. Malignant disease
  2. Oral contraceptive
  3. Pregnancy
  4. Postoperative or prolonged recumbency
  5. DIC
  6. Lipid
  7. Antithrombin III deficiency
  8. Protein S and C deficiency

### Therapy and Prophylaxis

#### 1. Anticoagulants

- Low molecular weight heparin: In this type of heparin decreased incidence of heparin induced thrombocytopenia and no test required.
- Unfractionated heparin- maintains PTT 1.5-2.5 X the normal control.
- Coumadin (Warfarin)

#### 2. Thrombolytics

- Plasminogen activators
- Snake venom enzymes (ancrod)

#### 3. Antiplatelet agents

- ASA
- Sulfinpyrazone
- Dipyridamole



## REVIEW QUESTIONS

1. Factor I is commonly known as
  - a. Tissue factor
  - b. Prothrombin
  - c. Antihemeophilic factor
  - d. Fibrinogen
  
2. The traditional intrinsic coagulation pathway includes factors
  - a. X, V, IV, III, II and I
  - b. XII, XI, IX, VIII, Prekallikerin and HMWK
  - c. XII, X, IX, XIII, Prekallikerin and HMWK
  - d. VII and tissue factors
  
3. A substance released from platelets that promotes their aggregation is
  - a. HMWK
  - b. ADP
  - c. Factor V
  - d.  $\alpha_2$ -antiplasmin
  
4. Epistaxis is mean
  - a. Nose bleed
  - b. Vomiting of blood
  - c. Excessive menstrual bleeding
  - d. Blood in the urine
  
5. A normal hemostatic mechanism depends upon the normal structure and function of
  - a. Tissue surrounding the blood vessels
  - b. Platelets
  - c. Blood vessels
  - d. Plasma coagulation protein
  - e. All of the above
  
6. The coagulation factors that are vitamin K dependent are:
  - a. I, V, VIII and XIII
  - b. II, VII, IX, and X
  - c. XII, XI, Prekallikerin
  - d. II, VII, IX and XI
  
7. Thrombin has many roles in hemostasis
  - a. Activation of factor XIII
  - b. Activation of protein C
  - c. Conversion of fibrinogen to fibrin
  - d. Both enhancing and inhibiting coagulation
  - e. All of the above

8. Antithrombin III inhibits

- a. Factors IIa, IXa, and Xa
- b. Plasmin
- c. Factors XIa and XIIa
- d. Kallikrein
- e. All of the above

9. The Prothrombin time test

- a. It is the most frequently used test to monitor anticoagulant therapy with Vitamin K antagonists
- b. It is a good screen for the intrinsic and common pathways.
- c. It is reported as a percentage of factor activity
- d. All of the above

10. The Thrombin time test will be prolonged by all of the following except:

- a. Dysfibrinogenemia
- b. Fibrin Split Products
- c. Elevated fibrinogen levels
- d. Heparin

11. The international normalized ratio (INR) is useful for

- a. Determining coagulation reference ranges
- b. Monitoring heparin therapy
- c. Monitoring thrombotic therapy
- d. Monitoring warfarin therapy

12. The international normalized ratio (INR) is used to correct for differences in reagent preparations for

- a. Prothrombin time
- b. Partial Thromboplastin Time
- c. Activated Clotting Time
- d. Lee-White Clotting time.

13. Which of the following is NOT a vitamin K dependent factor?

- a. Factor V
- b. Factor VII
- c. Prothrombin
- d. Factor IX
- e. Factor X

14. Which vitamin K dependent clotting factor has the shortest half-life?

- A. Prothrombin
- B. Factor VII
- C. Factor IX
- D. Factor XI

15. Which of the following factor deficiencies would be expected to result in prolongation of both the Prothrombin Time (PT) and the Partial Thromboplastin Time (PTT)?

- a. Factor XI
- b. Factor X
- c. Factor IX
- d. Factor VIII
- e. Factor VII

16. Which of the following factors is least likely to be depleted in disseminated intravascular coagulation (DIC)

- a. Fibrinogen
- b. Factor IX
- c. Factor VIII
- d. Factor V
- e. Factor X

17-22: Correlation

17. Usual pattern of inheritance is sex-linked recessive

18. Associate with decreased factor VIII procoagulant activity

19. Associated with decreased factor VIII procoagulant activity and normal bleeding T

20. Associated with both autosomal dominant and recessive inheritance

21. Associated with decreased factor IX procoagulant activity

22. Associated with a prolonged bleeding time and defective ristocetin platelet aggregation

- A. Hemophilia A
- B. Hemophilia B
- C. VonWillebrand
- D. A and B
- E. A and C

23. With regard to anticoagulant therapy

- a. Warfarin is safer than heparin in pregnancy
- b. The INR is used to control heparin therapy
- c. Vitamin K is used to reverse the action of warfarin
- d. Vitamin C is used to reverse the action of heparin

24. All of the following about platelet are correct except:

- a. Are often multinucleated
- b. Are often increased in number
- c. Platelets increased in patients with IDA caused by chronic blood loss
- d. Are sometimes reduced in number in vonWillebrand disease.

25. The following are correct in DIC except

- a. Is commonly seen as a presenting feature of AML (M3)
- b. Is usually associated with reduced fibrinogen levels
- c. Is usually associated with raised platelet count
- d. Is usually associated with a prolonged APTT



Blood transfusion is an important part of the treatment of many malignant and non-malignant hematologic disorders. Children with thalassemia, adults with myelodysplastic syndromes, and patients with autoimmune hemolytic anemias, leukemias, or aplastic anemias are depending on blood transfusions. Modern treatment procedures like high-dose therapy and stem cell transplantation

**BLOOD GROUPS**

Human red cell contains on their surface a series of glycoproteins and glycolipids, which constitute the blood group antigens. Approximately 400 RBCs antigens have been described. The development of these antigens is genetically controlled; they appear early in fetal life and remain unchanged until death. On the basis of these antigens, at least 15 well-defined red cell blood group systems of wide distribution in most racial groups have been described. They are the ABO, MNS, P, Rh, Lu, Kell, Lewis, Duffy, Kidd, Diego, Yt, Xg, Li, Dombrock and colton systemt.

The antibodies to the red cell antigens are of two types:

1. Naturally occurring antibodies occur without any obvious antigenic stimulus in the serum of individual lacking the corresponding red cell antigens, the iso-agglutinins of the ABO system are the main example.
2. Immune or acquired antibodies: are produced in individuals as a result of stimulation by a red cell antigen which is not present on their own red cell or in their body fluids. This antigenic stimulation may arise from blood transfusion or the result of pregnancy.
3. Complement-binding antibodies: Both naturally occurring.

The interaction of red cell blood groups and antibodies directed against these antigens usually is detected by agglutination methods in the blood bank. Agglutination of red cells can be considered to have two distinct phases: the first (sensitization) where antibody makes physical contact with antigen, and the second (agglutination) where antibody coated cells interact with each other. Some antibodies, particularly those of the IgM class, can cause agglutination of red cells directly; their pentmeric structure is sufficient to bridge the distance between cells and has led to their description as complete antibodies.

Temperature is important in blood group serology. Some antibodies are considered "warm and react best at 37° C; these usually are the most important in transfusion and the most likely to cause adverse transfusion reactions. Other antibodies react best in the cold.

Blood group antibody attachment can be affected by pH as well. For example, some antibodies reactive against the M antigen are enhanced at pH 5.5, whereas others are best detected at neutral pH.

The ratio of antibody to antigen also can affect the rate of antibody attachment in the red cell sensitization

## The ABO Blood Groups

The ABO system was the first blood group recognized, and remains pre-eminent in blood transfusion practice. In 1900, Landsteiner tested red cells and sera from his laboratory workers and noted that the sera from some workers agglutinated the cells of others but not their own. He divided individuals into three groups (A, B, and O) based on these experiments, and in 1902, Von Decastello and Sturli found the fourth group, AB. The blood group classified into four main groups, AB, A, B and O which are determined by the presence or absence on the red cells of two antigens A and B. the frequency of the ABO groups differs in different geographical regions but in Yemen it is approximately; group O 49%; A30%; B18% and AB 3%.

Transfusion of ABO-incompatible blood results in an acute hemolytic reaction which be life threatening.

The antigens are under the control of three allelic genes, A, B and O situated on the long arm of chromosome 9 and are inherited in a simple Mendelian fashion.

**Table 18.1: Blood groups**

Name of blood group	Genotype	Antigen present in the RBC	AB normally in serum	AB occasionally present
A <sub>1</sub> B	A <sub>1</sub> B	A+A <sub>1</sub> +B	Nil	Anti-H
A <sub>2</sub> B	A <sub>2</sub> B	A+B	nil	Anti-A <sub>1</sub>
A <sub>1</sub>	A <sub>1</sub> A <sub>1</sub> , A <sub>1</sub> O	A+A <sub>1</sub>	Anti-B	Anti-H
A <sub>2</sub>	A <sub>2</sub> A <sub>1</sub> , A <sub>2</sub> O	A	Anti-B	Anti-A <sub>1</sub>
B	BB, BO	B	Anti-A+A <sub>1</sub>	Anti-H
O	OO	neither A nor B	Anti-B Anti-A Anti-A <sub>1</sub>	none

The basic substance is called H antigen, and it is postulated that its formation is controlled by another gene (H), which is separate from the ABO Genes. H antigen is present on all red cells (except Bombay cells) but the amount formed is influenced by the A and B genes, the action of which is to convert some of the H antigen to A and B antigen.

The A subgroups; several subgroups of A exist, the most important being A<sub>1</sub> and A<sub>2</sub>.

Group AB has similar subgroups-A<sub>1</sub>B and A<sub>2</sub>B.

Approximately 20% of group A and group AB subjects belong to group A<sub>2</sub> and A<sub>2</sub>B respectively; the remainder belong to group A<sub>1</sub> and A<sub>1</sub>B, Substances with antigenic properties closely similar to those of A and B are widely distributed in nature and are found in many animals and bacteria. Absorption of the substances from the gut is presumed to give rise to the production of anti-A and anti-B in the plasma of those who do not passes the substances on their red cells. Because of the presence of these antibodies it is necessary to transfuse blood with the same group as that of the recipient. As group O cells do not either with anti-A and anti-B, people of group O came to be known as universal donors. However, this is a

dangerous concept, because group O people have anti-A and anti-B in their plasma, and in a small number of people these antibodies may be very potent so that a transfusion of 500 ml of group O blood may contain sufficient anti-A or anti-B to react with the recipients cells and bring about their destruction. Group O blood should be given to group A or B people only if the correct group is not available or if there is insufficient time to find out the group of the recipient.

### **The Rhesus (Rh) Blood Groups**

The awareness of the blood group system termed Rh began in 1939, when Levine and Stetson published a case where the mother of a stillborn baby had a transfusion reaction after receiving the husband's ABO-compatible blood. It was found that some human red cells were agglutinated by the serum- Rh-positive cells, while others were not agglutinated- Rh-negative cells. Rh is the second clinically important and complex blood group system.

In 1940, Landsteiner and Weiner found additional evidence when they discovered that sera raised in rabbits and guinea pigs who had been injected with rhesus monkey red cells would agglutinate rhesus cells.

#### **Antigens**

Rh consists of more than 48 antigens but it is only determined by three pairs of closely linked allelic genes located on chromosome 1, C or c, D or d, E or e. are important. After discovery of Rh system in 1940, various theories were postulated to explain the mode of inheritance and different nomenclature were proposed. The World Health Organization in 1977 recommended the CDE nomenclature of Fisher. Cde (with R1 as short symbol) are the most common haplotypes in caucasians

One set of the three genes is inherited from each parent, giving rise to various combinations of genotypes, e.g CDe from one parent and CDe from other, with the resulting genotype CDe/CDe

- cde/cde      Negative
- Cde/cde      Negative
- CDe/cde (+), cDe/cDe (+), cDe/cde (+), CDe/cDe (+)
- cDE/cDE (+), others (+).

D is a strong antigen, and is by far the most important. In clinical practice, Rh grouping is performed with an anti-D antiserum; Persons who are D positive are referred to as Rh positive, and those who are D negative is Rh negative.

Approximately 83% of the population is Rh positive and 17% is Rh negative.

The Rh antigen is found in association with red cell membrane protein of unknown function. The U, LW and Duffy blood group antigens are thought to be associated with the same protein. In contrast to the ABO and Lewis, Rh antigen does not contain sugars.

#### **Antibodies**

Practically all Rh antibodies result from immunization, naturally occurring Rh antibodies, with exception of anti-E, are rare. Immunization may result from the transfusion of Rh-positive blood into an Rh negative person, or



from the passage of Rh positive cells from a fetus into the circulation of an Rh negative mother during pregnancy.

When an Rh-negative person has been immunized either by a transfusion or pregnancy, the transfusion of Rh-positive blood can result in a hemolytic transfusion reaction, which may be fatal.

D is a strong antigen and thus a large proportion of Rh negative persons exposed to Rh-positive cells become immunized.

Transfusion constitutes a more effective stimulus than pregnancy. The antibody to the D antigen (anti D) may occur in two forms: as a saline agglutinating antibody (IgM) and as an incomplete antibody (IgG) the latter is the more common.

The other antigenic of the Rh system are much less antigenic than D, and thus are of less clinical importance. However, occasionally anti E, anti C, anti-c and anti-e develop as a result of transfusion or pregnancy; they may develop in D-positive patient. Their presence can be detected by careful cross matching; their identification requires special laboratory investigation.

**Table 18.2: RH antigens**

Genotype	Designation	Phenotype	Rh D status	Frequency %
CDe/cde	R <sup>1</sup> r	Cc De	Positive	32
CDe/Cde	R <sup>1</sup> R <sup>1</sup>	CDe	Positive	17
Cde/cde	Rr	Ce	Negative	15
CDe/cDE	R <sup>1</sup> R <sup>2</sup>	Ce DEe	Positive	14
cDE/cde	R <sup>2</sup> r	cDEe	Positive	13
cDE/cDE	R <sup>2</sup> R <sup>2</sup>	cDE	Positive	4

Most Rh antibodies are IgG immunoglobulins, which are produced in response to contact with foreign Rh antigens as a result of blood transfusion or pregnancy. Because the most immunogenic of the Rh antigens is the D antigens, antibodies with anti-D specificity are particularly common.

#### OTHER BLOOD GROUP SYSTEMS

**The Kell blood group system**, is common with the Rh system, is under the control of the closely linked alleles K and k, kp<sup>a</sup> and kp<sup>b</sup> and JS<sup>a</sup> and JS<sup>b</sup>. The synthesis of the Kell antigens is incompletely understood but most workers currently believe that a gene on the X-chromosome encodes a Kell precursor antigen, Kx, which is subsequently converted to the final Kell antigens.

The Kell group system is clinically important because Kell antigen, are highly immunogenic and antibodies with Kell system specificity have been implicated in hemolytic transfusion reactions and hemolytic disease of the newborn. The most commonly encountered immune antibody outside of the Rh system is anti-K.

**The Duffy blood group system** is encoded by a number of alleles located on chromosome 1. The Duffy antigens include Fy<sup>a</sup> and Fy<sup>b</sup>. Interestingly, the Duffy phenotype plays an important role in determining susceptibility to certain forms of malaria. The Duffy antigens are associated with the surface receptor, which facilitates invasion of the red cell by the malarial parasite plasmodium vivax.

Individuals with the Fy (a-b) phenotype are highly resistant to plasmodium vivax infection.

**The Lewis** blood group system has been described in conjunction with ABO. Both anti-Le<sup>a</sup> and anti-Le<sup>b</sup> can occur naturally and rarely are of clinical concern. Because these antibodies usually are IgM and cannot cross the placenta and do not cause hemolytic disease of the newborn.

**The P System** has been characterized biochemically largely due to its presence in hydatid fluids. It rarely is related to transfusion problems unless the patient is of the rare P phenotype

**The MNSs System** is a complicated blood group whose biochemically is unfolding under current investigation. Certain antibodies to its antigen (anti-M, anti-n) usually occur naturally and are unlikely to produce transfusion difficulties; anti-S, -s and -U, usually are immune antibodies and can cause both hemolytic disease of the newborn and transfusion reaction

The Li system differs from other blood groups in several ways. The I antigen is abundant on fetal and newborn red cells, but I antigen strength increases gradually and becomes predominant before 2 years of age.

#### **Screening for unexpected RBC**

It is done routinely on each specimen submitted for blood grouping. Unexpected Abs are specific for RBC blood group Ags other than A and B e.g Rh (D), Kell (K), Duffy(Fy), and Hr. (c). Early detection is important because such Abs can cause hemolytic disease of the newborn and serious reactions and they greatly complicate and delay compatibility testing (cross-matching) and procurement of compatible blood.

#### **White Cell Group**

The most readily recognised antigens on the surface of granulocytes and lymphocytes belong to the HLA (Human Leukocyte Antigen) system. This system has assumed great clinical importance in recent years with demonstration that the same antigens are present on the nucleated cells of many body tissues and act as transplantation antigens.

#### **The HLA System**

The antigen of the HLA system are determined by allelomorphic genes at six closely linked loci, designated HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP, situated along a segment of chromosome 6 referred to as the HLA region.

#### **Platelet Groups**

ABO and HLA-A, HLA-B, and HLA-C antigens are found on the surface of platelets and a number of platelet-specific antigens have also been demonstrated. Platelet antibodies may be detected in patients who have received multiple blood or platelet transfusions and in multiparous female. The incidence of Allo-immunization in leukemic patients receiving prophylactic platelet transfusions reaches 50% in some series, and the resulting shortening of the survival time of the transfused platelets may cause considerable problems.

Platelet specific antigens and antibodies are usually defined by test using fluorescein-Labelled anti-immunoglobulin serum.

### Cross- Matching

After the blood group and Rh typed of the patient have been established and a donor of the same blood group and Rh type has been tentatively selected, cross-matching tests must be made. That is to say, one must match the donor cells with patient's serum and the patient's cells with donor's serum, and there must be no signs of agglutination of the red cells.

The compatibility test is carried out for two purposes:

- A) To guard against a mistake in ABO or Rh grouping.
- B) To demonstrate immune antibodies in the patient serum, active against the donor cells.

As no single test is capable of disclosing all types of incompatibility satisfactory, four tests are recommended:

1. A saline test carried out at room temperature.
2. A saline test carried out at 28 – 30 °C
3. On albumine test carried out at 37°C
4. On indirect antiglobulin test (coomb's test) sensitising the cell at 37°C

The saline test is carried out at room temperature, 20-25°C rather than 37°C because antibodies react better at lower temperature. However, several cold antibodies of specificity other than anti A or Anti B may be also cause agglutination at room temperature.

If agglutination is observed at 20-25 °C the test at 28-30°C should be read and provided the indirect antiglobulin test and the albumin test are negative, the blood issued as compatible if it is needed.

Bovine Albumin: This method will picked up some incompatibilities not detected in saline due to incomplete, immune antibodies, such as Rh antibodies.

Blood of the same ABO and RH D group is selected. The cross-match normally takes about 40 minutes in normal ionic strength saline (NISS). When blood is required urgently, the tests may be carried out quickly by limiting the tests performed and modifying the techniques. This reduces test sensitivity but will detect all gross incompatibilities. Transfusion of un-cross-matched blood in emergencies carries considerable risk and should be avoided when possible. When the urgency of a clinical situation does not allow time for grouping the patient, group O Rh-negative blood should be transfused. Two units of group-checked blood are kept for emergency situation.

*All donors and recipient may be regrouped or retyped whenever they appear for examination or transfusion, and every patient or donor must be re-examined before every transfusion*

### BLOOD TRANSFUSION

The most important indications of transfusions are to restore blood volume and treat shock following acute blood loss, and to provide RBCs to maintain blood Hb level. Individual blood components such as RBCs, platelets, WBC, whole plasma, or specific plasma proteins may be used effectively instead of whole blood.

The standard blood donation is 450 ml, taken into a plastic bag that contains adenine supplemented cit-phosphate-dextrose (CPDA-1) blood maybe stored for 35 days. Stored whole blood differs considerably from circulating blood.



A number of laboratory tests must be completed before blood or blood products can be transfused: (1) Determination of the blood type with a crossmatch. (2) Screening for antibodies that may produce adverse effects if transfused. (3) Screening for possible infectious agents that could be transmitted with transfusion.

The following tests are mandatory on all units of blood collected for transfusion:

- (1) ABO group and Rh type
  - (2) Screening for blood-group antibodies
  - (3) Serologic test for syphilis
  - (4) Serologic tests for human retroviruses including: HIV-1 antibody, HIV-2 antibody, HIV p24 antigen, HTLV I antibodies
  - (5) Serologic tests for hepatitis including hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb) and hepatitis C antibody
- If, all of these markers are negative can blood be conveyed to the Blood Bank for storage until usage. Positive results for some of these tests may prevent further donation by that person. A person with such a test result will be notified by the donor center.

No IV solution other than 0.9% sodium chloride solution should be allowed into the blood bag or in the same tubing with blood, since many solutions exert deleterious effects (e.g D/W causes clumping and decreased survival of RBCs; Ringers solution causes clotting).

Before any transfusion is started, both label and report of compatibility testing must be checked to ensure that the blood is indeed for the patient concerned, that it is compatible, and the component is correct.

### **Indication of Transfusion**

#### **1. Acute hemorrhage**

Acute loss of 15-20% or more of circulating blood volume leads to tachycardia, hypotension, +/- shock in addition to weakness and syncope.

The most common causes:

- a. Traumatic hemorrhage or surgical operations.
- b. Hemostatic defects leading to severe hemorrhage.
- c. GIT bleeding due to oesophageal varices, peptic ulcer, Meckel diverticulum.

Severe hemorrhage results from the umbilicus.

#### **Treatment**

- Local measures to control hemorrhage.
- Whole blood transfusion: Urgent 20 ml/kg to restore blood volume and treat shock.
- Plasma or plasma expanders may be used to sustain the shocked patient till blood become available.

#### **2. Chronic Anemia**

Slowly developing anemia is less dangerous than acute blood loss, and symptoms may be minimal despite markedly low Hb.

If due to specific factor deficiency, treatment should be directed to the cause as iron, folic acid, and vitamin B12.

Blood transfusion is not indicated except if anemic heart failure or infections are present.

In progressive refractory anemia as thalassemia major and pure red cell anemia, packed RBCs especially leukocyte poor or glycerol frozen

preparation are preferred. Febrile reactions are decreased due to reduction of antigens.

The dose of packed RBCs is 10 ml/kg body weight. In severe anemia, small repeated doses (2-4 ml/kg) of packed RBCs are preferred with simultaneous use of lasix.

In case of anemic heart failure, a modified exchange transfusion is done, replacing the patient's blood by packed RBCs.

Intra-uterine blood transfusion indicated in case of severe Rh incompatibility occurring before 32 - 34 weeks gestation.

Exchange transfusion is indicated in hyperbilirubinemia, septicemia, RDS and Reye syndrome.

3. Providing deficient factors in hemostatic disorders as purpura, hemophilias etc.

4. Raising plasma protein concentration in hypoproteinemias as nephrotic syndrome, nutritional anemia, and cachexia.

5. Providing antibodies in case of severe infections, septicemia.

6. Providing WBCs in leukopenia and agranulocytosis.

### **Human Blood Products**

#### **1. RBCs: In case of chronic anemia**

- Packed RBCs
- Red cells suspended in an appropriate solution.
- Leukocyte poor RBCs
- Saline washed RBCs
- Reconstituted frozen RBCs

#### **2. Platelet Transfusion**

Platelet transfusion used to stop bleeding in case of hemorrhage due to thrombocytopenia ( $< 20000/\mu\text{L}$ ). It is more useful in cases of inadequate production as hypoplastic pancytopenia and leukemia than into peripheral hyperdestruction as ITP:

- Fresh platelet rich plasma
- Platelet concentrates
- Pooled platelets (from multiple donors)
- ABO and Rh compatible platelets are usually used. HLA compatible platelets are superior as they evoke no antibody response and give a more satisfactory platelet survival.

*"One platelet unit increases the count by  $10000/\mu\text{L}$  in adult"*

#### **3. Granulocytes Transfusions**

It is of limited therapeutic use, due to short half-life and technical difficulties. Granulocytes are collected by cytopheresis and given within 24 h of harvest. It is indicated in (1) Leukopenic patient with gram-negative sepsis and (2) Febrile patients with neutropenia resulting from cancer chemotherapy, or bone marrow transplantation.

#### **4. Plasma and Plasma Components**

a. **Whole plasma:** Plasma components used to restore plasma volume in case of acute dehydration are:

- Fresh plasma
  - Fresh frozen plasma
  - Dried lyophilized plasma.
- b. Human salt free albumin: in case of hypoproteinemia
- c. **Clotting factors**

- Factors VIII preparations indicated for treatment of Hemophilia and VW disease
- Proplex or Konyne (factor II,VII,IX,XI) is indicated for treatment of hemophilia B
- Human fibrinogen.
- d. **Immunoglobulin:** Human Gamma globulin preparations: used by IM injections.  
Recently, intravenous Gamma Globulin is used.

## **Complication of Blood Transfusion**

### **I. Complication Appearing Early**

#### **1. Hemolytic transfusion reaction:**

Usually due to ABO incompatibility

Clinical feature: Fever, rigors, lumbar pain, dyspnea, hypotension, hemoglobinuria and acute renal failure. In severe cases; DIC may occur, with very bad prognosis.

Diagnosis: By clinical features, repeat cross matching, and recheck labels on blood containers.

#### **Treatment**

Stop transfusion

Repeat cross matching

Examine urine and plasma for free Hb

Diuresis by fluid therapy and Mannitol should be started.

If the period of renal failure could be managed adequately, recovery occurs.

#### **2. Allergic reaction**

About 1-2% of transfusions are leading to urticaria, itching+/- wheezing and arthralgia. It may be due to antibodies in donor's plasma.

Treatment: Antihistamines or steroid.

#### **3. Febrile reactions: due to**

A. Pyrogenic reaction due to pyrogens in the equipment used is less common now. It can be controlled by antipyretics.

B. Sensitization to WBCs antigen, which cause fever flushing and chills. It is caused by antibodies to donors WBCs. It can be avoided by use of washed or leukocyte-poor packed RBCs, or use of antipyretics.

C. Infected blood: may lead to fever, shock and death.

#### **4. Circulatory overload: due to excessive amount of speed of transfusion.**

5. Air embolism

6. Thrombophlebitis

7. Citrate toxicity

8. Hyperkalemia

### **II. Complication Appearing Late**

#### **1. Transmission of Diseases**

a. Hepatitis: Screening all donors for Hbs-Ag minimized the risk of transmission of serum hepatitis. Recently, the most common type of hepatitis is following transfusion of hepatitis C virus.

b. AIDS caused by HIV, screening for the antibodies against the virus by ELISA.

c. CMV which may lead to severe pneumonia, hepatitis, thrombocytopenia and hemolytic anemia in the prematures receiving blood from seropositive donors.

d. Other viruses include EBV and HTLV-1 viruses



- e. Parasites: Malaria, toxoplasmosis and trypanosomiasis.
- f. Syphilis.

**2. Red Cell Isoimmunization:** due to RBCs minor group antigens.

**3. Sensitization** to WBC, platelets and plasma protein antigen of these lead to allergic reactions in the next transfusions. Also, trans-placental passage of formed IgG may lead to isoimmunization, neonatal ITP.

**4. Iron overload:** Each 500 ml blood contains about 200 mg iron. Therefore repeated prolonged transfusion therapy may lead to hemosiderosis as in thalassemia.

**5. Graft-versus-Host-Disease (GVHD)** rarely occurs, especially in immunodeficient patients transfused by fresh blood containing immunocomponent WBC.

### Investigations in a Case with Transfusion Reaction

In a case develops features suggesting a severe transfusion reaction, the transfusion should be stopped and investigations for blood group incompatibility and bacterial contamination of the blood must be initiated.

1. Most severe reaction occurs because of clerical errors in the handling of donor or recipient blood specimens. Therefore it must be established that the identity of the recipient is the same as that stated on the compatibility label and that this corresponds with the actual unit being transfused.
2. The unit of donor blood and post-transfusion samples of the patient's blood should be sent to the laboratory that will:
  - (a) Repeat the group on pre- and post-transfusion samples and on the donor blood, and repeat the cross-match
  - (b) Perform a direct antiglobulin test on the post-transfusion samples
  - (c) Check the plasma for hemoglobinemia
  - (d) Perform test for DIC
  - (e) Examine the donor sample directly for evidence of gross bacterial contamination and set up blood cultures from it at 20°C and 37°C
  - (f) Urine must be examined for hemoglobinuria.

The following observations are helpful for continuation your investigations:

- (1) If there is no evidence of free hemoglobin or increase in bilirubin, it is not likely there has been any serious degree of hemolysis.
- (2) The presence or absence of oxyhemoglobin methemalbumin or increased bilirubin in the plasma naturally depends on the rate at which the blood was being transfused before the sample was taken and the rate of hemolysis.
- (3) If it seems possible that the hemolysis was due to serological incompatibility, the next step is to try to find the cause
- (4). If the compatibility test is clearly negative, it is unlikely, although not impossible, that the hemolytic reaction is due hemolysis of the donor's red cells. The next step is to consider the possibility that hemolysis of the patients red cells has taken place, perhaps due to immune anti-A (or anti-B) being transfused in group O blood given to group A (or B) recipient. The

Anti-A (or Anti-B) titre of the transfused plasma should be ascertained and tests carried out for Anti-A (or B) hemolysis and for incomplete antibodies. If group O blood has been given to a group A or B recipient osmotic fragility of the recipient blood should be measured and blood film stained. An increase in osmotic fragility and the presence of spherocytes are pointers to hemolysis reaction being due to the transfusion of immune anti-A or anti-B.

(5) In the absence of positive findings, the patient's serum is examined 5-10 days later for red or white cell antibodies.

## APHERESIS

The process of apheresis involves removal of whole blood from a patient or donor. Within an instrument that is essentially designed as a centrifuge, the components of whole blood are separated. One of the separated portions is then withdrawn and the remaining components are retransfused into the patient or donor.

The components which are separated and withdrawn include:

1. Plasma (plasmapheresis)
2. Platelets (plateletpheresis)
3. Leukocytes (leukapheresis)

## Therapeutic Apheresis

The purpose of therapeutic apheresis is to remove a component of the blood which contributes to a disease state. Examples include:

- Plasmapheresis: within the plasma are contained antibodies and antigen-antibody complexes that may contribute to the deleterious effects of autoimmune diseases. Removal of the plasma (and replacement with saline solution) will help to reduce circulating antibodies and immune complexes.

In rare circumstances, excess blood proteins are present that may cause circulatory problems. Examples of these diseases include:

- ✚ Waldenstrom's macroglobulinemia
  - ✚ Myasthenia gravis
  - ✚ Guillain-Barré syndrome
  - ✚ Hyperviscosity Syndromes
  - ✚ Paraproteinemia
  - ✚ Cryoglobulinemia
  - ✚ Goodpasture's syndrome
- Plateletpheresis: rarely, in myeloproliferative disorders, the platelet count can be very high (thrombocytosis). Removal of platelets can help to avoid complications of thrombosis and bleeding.

- Leukapheresis: in some cases of leukemia with very high white blood cell counts, removal of the excess leukocytes may help to prevent complications of thrombosis.
- Stem Cell Harvesting: the small number of circulating bone marrow stem cells can be harvested to use in transplantation procedures.

## REVIEW QUESTIONS

1. A group O woman has given birth to a group O baby. All of the following men could be the fathers except.
  - a. Hamad, who is group O
  - b. Shad who is group A (genotype AO)
  - c. Dader, who is group AB
  - d. Salem, who is group B (genotype BO)
2. The followings are correct about severe hemolytic transfusion except:
  - a. Can always be prevented by invitro testing
  - b. Are usually due to human failure to follow established procedure
  - c. Occur relatively more often in patients previously transfused or exposed to blood.
  - e. Often cause acute tubular necrosis
3. Rh negative mothers may have been sensitised to Rh-positive blood of the following except:
  - a. A second trimester abortion
  - b. A childhood blood transfusion
  - c. Being an Rh negative child of an Rh positive mother
  - d. A previous pregnancy with an Rh negative baby
4. Fresh plasma is the component of choice in the management of:
  - a. Factor V deficiency
  - b. Factor VII deficiency
  - c. Hemophilia
  - d. Factor X deficiency
5. The risk of transfusing blood containing hepatitis B surface antigen as compared to blood negative for this is:
  - a. Increased
  - b. Decreased
  - c. Increased only in patients who have not previously transfused
  - d. Identical
6. Massive transfusion of stored whole blood has been shown to be associated with:
  - a. Change in Acid-Base balance
  - b. Alteration of hemoglobin-Oxygen affinity
  - c. Hypocalcemia and hyperkalemia
  - d. Clotting deficiencies
  - e. All of the above



7. Platelet transfusion should be given:
- When the platelet count is less than 20000
  - When the patient bleeding and the platelet count less than 20000 due to hypoplasia
  - In cases of drug purpura
  - In DIC
8. Stored plasma (4 for 21 days) is suitable for replacement of all the following except:
- Prothrombin
  - Factor VIII
  - Factor IX
  - Factor VII
9. Blood group testing
- Can establish maternity
  - Can exclude maternity
  - Can occasionally establish paternity
  - Can exclude paternity
10. The presence of the most immunogenic of the Rh antigens leads to the designation of a patient as being Rh positive. Which of the following antigens is responsible?
- C
  - D
  - E
  - e

Q11. It is apparent that any one's ABO blood groups can be determined by testing his red cells for the presence or absence of the two agglutinogens, A and B. Where can one readily obtain suitable reagents to perform such tests? Show in tabular form the reactions to be expected for each of the four blood groups, when such reagents are used.

A11. The two reagents required are anti-A serum and anti-B serum. Obviously the serum of a group B person can serve as the anti-A reagent, and the serum of a group A person as the anti-B reagent.

Blood specimen	Reaction of the red cells with		Diagnosis of blood group
	Anti-A serum	Anti-B serum	
1	-	-	O
2	+	-	A
3	-	+	B
4	+	+	AB

Q12. Group O individuals are at times used as "universal donors" i.e. their blood is used for transfusing patients of all four blood groups; similarly, group AB patients are at times treated as universal recipients" on what principles is that practice based? What are the limitations of the use of group O as universal donors and of group AB as universal recipients?

A12. Since group O red cells are inagglutinable by anti-A or anti-B the chief sources of danger when using group O blood for agglutinins in the donors' serum. However, the donor's agglutinins are highly diluted in the recipient's circulation, and also tend to be neutralized by the blood group substances present in the organs and secretions of group A, B or AB recipient. The same applies when group AB patients are given blood of group O, A, B. the danger of this practice is that some group O donors have very high isoantibody titers which may insufficiently be diluted and neutralized

## BONE MARROW TRANSPLANTATION

# 19

High dose chemotherapy or chemoradiotherapy followed by bone marrow transplantation is now used to treat a wide range of hematological malignancies and has also show promise recently in the management of certain non-malignant conditions such as beta thalassemia. The science of bone marrow transplantation is advancing rapidly and provides a true forum for many different specialities- hematology, oncology, immunology, radiotherapy as well as bacterial and antiviral chemotherapy. There are accounts of the increasing success being achieved using high dose chemotherapy in association with autologous bone marrow transplantation, and the management of viral infections (particularly the herpes group) in immunosuppressed patients.

### Principle of Bone Marrow Transplantation

Many types of tumors in man show a dose response relation to anti-neoplastic drugs and radiation, but the dose that can be given is limited primarily by toxic effects on the bone marrow. This limitation can now be circumvented by IV infusion of normal hematopoietic stem cells.

Three sources of hematopoietic cells have generally been utilized:

1. Syngeneic bone marrow from a genetically identical twin.
2. Allogeneic bone marrow from a histocompatible sibling donor.
3. Autologous cryopreserved stem cells of the patient either collected from his bone marrow or peripheral blood.

### Indication of Bone Marrow Transplantation

Although most marrow transplants are for hematologic malignancies, the most common indications for allogenic and autologous transplants differ.

1. Allogenic transplants about 73%, Leukemia 28% for CML, 24% for AML; 20% for ALL and 1% for other malignancies or premalignant disorders, including NHL, Hodgkin's disease 1%, myelodysplasia 4%, neuroblastoma 4%, multiple myeloma 1%, Aplastic anemia 8%, immune deficiencies 2%, and inherited disorders of metabolism 1% .
2. Autologous BMT : is used only for malignancies, NHL 25%; Breast cancer, 24%; Hodgkin's disease 17%; AML, 11%; Multiple myeloma, 4%; ALL, 3%; Neuroblastoma 3%; and other Cancers, 13%.

### Selection of Donor

1. Fully matched sibling: Matching is performed by serological typing of class I and Class II MHC, as well as, bidirectionally negative mixed lymphocyte culture.
- (2). Other family donors: Including parents or other sibling non- identical in one A or one B class I MHC locus. For such cases typing of class II MHC has to be performed both serologically and at the DNA level.



## Evaluation of Recipient

Prior to transplant, dental, ENT and gynecological examinations are done for all patients to exclude septic foci. Liver and kidney function tests, respiratory function tests, ECG, Echocardiography, chest X-ray and abdominal sonography are performed to exclude any organ function impairment. Serological tests for toxoplasmosis and the following viruses: HBV, CMV, HIV, HCV, EBV, HSV, and HZV are also performed. Patients with recent infections are excluded.

## Procedure

1. Isolation: Prior to isolation, a Hickman right atrial catheter is inserted. All patients are nursed under strict protective isolation in either horizontal or vertical laminar air-flow units.

For total decontamination of the GIT non-absorbable antimycosis in combination with an aminoglycoside are given in addition to food sterilization. These measures are started 2 weeks before BMT and maintained for a minimum of 40 days post-BMT.

2. Preparative regimens: For untransfused severe aplastic anemia, cyclophosphamide is given in a daily dose of 50 mg/kg/day on 4 successive days. Polytransfused cases receive total nodal irradiation (4X2.5 Gy) in addition.

Malignant cases receive cyclophosphamide 60 mg/kg for 2 days in combination with either Busulfan in a total dose of 2 mg/kg on 4 successive days. For leukemia cases receive cyclophosphamide 60 mg/kg X 2 days with 200 rad on day 1-3 or Busulfan 2 mg/kg for 4 days with cyclophosphamide 50 mg/kg for 4 days and without radiation.

For prophylaxis against graft versus host (GVHD) patients receive cyclosporine A, 3 mg/kg/day iv by continuous infusion starting day-1 and short course methotrexate 15 mg/m<sup>2</sup> day+1 and 10 mg/m<sup>2</sup> days +3, +6 and +11.

3. Marrow harvesting: The marrow is harvested on day 0 from the donor in the operating room under general anesthesia. Marrow aspiration is carried out from the posterior superior iliac spine by multiple needle insertions at 1 cm intervals and vigorous suction applied while the needle is rotated.

The marrow is collected in Boxter's marrow collection bags and passed through 500 and 200 micron filters successively to break up the aggregated particles and to remove any bony spicules.

Samples are taken for cell counting. A total nucleated cell count of  $2-5 \times 10^8$  /kg BW of recipient is finally transfused to the patient through a central venous line.

For autografting, the patient must first undergo stem cell collection either from the bone marrow or from the peripheral blood by leukopheresis using a blood cell separator. This is followed by cryopreservation of the marrow using a controlled rate biofreezer and 10% dimethylsulfoxide as cryoprotectant. The stem cells are then stored in vapour phase liquid nitrogen.

4. Superior therapy: Parenteral hyperalimentation is given to all patients starting from day +5 till oral food intake is possible. All blood products are irradiated with 15 Gy prior to transfusion. Leukocyte poor red cell transfusions are given from random donors to maintain Hb level around 10-12 gm/dl until erythropoiesis by engrafted marrow is adequate.

Platelet concentrates are also given from random donors to maintain the platelet count above 20000/ $\mu$ L.

5. Follow up: The following investigations are done 2 times per week starting by 0 and once per week after discharge: complete blood count, reticulocyte count, liver function tests, kidney function tests, electrolytes fasting blood sugar and cyclosporine A level in the blood.

Long term follow up of recipients includes detection of chimerism by either sex chromosome in sex mismatch or detection of associated disease markers e.g Ph<sup>+</sup> chromosome cytogenitically or by molecular biology.

### **Potential Complications**

#### **1. Acute GVHD**

Acute graft versus host disease (GVHD), presumably triggered by disparity between donor and recipient for polymorphic non-HLA determinants occurs in about 40% of allograft. The reaction manifests itself by skin rash, cholestatic jaundice and diarrhoea of different severity grades. Severe immunologic deficiency accompanies GVHD and death from infection or liver cell failure frequently results.

#### **2. Chronic GVHD**

This is a distinct clinical syndrome affecting approximately 30% of transplant recipients who survive more than 3 months. Two-third of affected patients has preceding acute GVHD but in one third it develops de novo.

The incidence of chronic GVHD also increases with recipient age (more than 40 years.) Clinical and pathological features are similar to those seen in several haematologic and autoimmune disorders such as progressive systemic sclerosis, systemic lupus erythematosus, Sjogren's syndrome and primary biliary cirrhosis.

#### **3. Opportunistic Infections**

The early post-transplant phase is characterized by infection with gm<sup>+</sup>ve and gm<sup>-</sup>ve bacteria, viral infections and fungal infections especially *Candida albicans* and *aspergillus fumigatus*. Most troublesome are the interstitial pneumonia that typically occurs between 30 and 100 days after BMT. Formerly, approximately 10% of this pneumonia was related to pneumocystis carinii, but prophylaxis with trimethoprim-sulphamethoxazole has largely eliminated this problem. Approximately 60% of pneumonia is associated with the finding of CMV, and more than one-half of these are fatal. The other 40% of the interstitial pneumonia are of unknown origin.

#### 4. Veno-Occlusive Disease

In 5% of patients transplanted for leukemia. This is more common in patients with pre-existing liver function abnormalities and in patients who received extensive previous chemotherapy.

### STEM CELL TRANSPLANTATION

Stem cell transplantation (SCT) is the use of hemopoietic stem cell (HSC) from a donor harvested from peripheral blood or bone marrow, to repopulate recipient bone marrow.

#### Principle of Stem Cell Transplantation

**Allogeneic** SCT involves transplantation of HSC from one individual to another. This is usually between two HLA matching individuals, most frequently siblings but, in their absence, volunteer and HLA matched unrelated donors (MUD) are increasingly being used. HLA matching includes class I (A,B tested serologically) and class II (DR tested serologically or by molecular typing). If the donor is an identical twin, the transplant is termed "syngeneic". Mini transplants in which the recipient receives immunosuppressive but not myeloablative therapy are currently being explored.

**Autologous** SCT utilizes the patient's own stem cells. These are harvested from the patient then used to repopulate the marrow after further high-dose chemotherapy and / or radiotherapy.

**Cord** blood transplantation utilizes fetal stem cells harvested at the time of birth from the umbilical cord. Cord blood stem cells also possess a few characteristics that make them particularly more advantageous over routine bone marrow transplants. These native cells have been shown to be extremely proliferative once transplanted and exposed to internal growth factors. Furthermore, the chances of a severe complication with routine marrow transplants (Graft-Verses-Host Disease) are extremely rare due to the inability of juvenile cord blood to mount an attack against the recipient

Allogeneic SCT is rarely performed in individuals >55 years of age, as it carries risk of treatment-related morbidity and even mortality (up to 5-10%) which increases with age. Autologous SCT may be performed more safely in older patients up to 70 years.

#### Procedure

Treatment with a hemopoietic growth factor (e.g G-CSF), combined in the case of autologous SCT with chemotherapy, e.g. high-dose cyclophosphamide, is used to mobilize HSC from bone marrow into peripheral blood, where they are collected by leukapheresis. Alternatively, HSC may be harvested from marrow by multiple bone marrow aspirations, performed under general anesthesia. Approximately  $2 \times 10^6$ /kg CD34 cells are needed. The recipient of an allogeneic or MUD transplant then receives immunosuppressive drugs to reduce the risk of graft vs. host disease (GVHD).



## Indication of Stem Cell Transplantation

Stem cell transplantation is performed for congenital immunodeficiency disorders, bone marrow failure states, hematological malignancies, and solid tumors for which dose-intensive chemotherapy has greater effectiveness than standard dose chemotherapy. Because of the substantial risk of toxicity associated with stem cell transplantation, it should only be applied to patients for whom the risk; benefit ratio is favourable. In general, patients should be in good physical condition with adequate pulmonary, renal, hepatic and cardiac function, and performance status. Stem cell transplantation should be delayed in patients with active infection if possible. Patients with a malignancy are more likely to benefit from stem cell transplantation if their disease is in remission or has responded to conventional therapy. The results of stem cell transplants depend on many factors, such as recipient's age, stem cell source, HLA identity between donor and recipient, type of disease, and disease stage. Details about the indication and the results of stem cell transplants are given in the chapters dealing with specific diseases.

**Table 19.1: Indications of stem cell transplantation**

Allogeneic	Autologous
Acute leukemia 1. Standard/poor risk AML in 1 <sup>st</sup> remission 2. AML in second remission 3. Poor risk childhood or adult ALL in first remission ALL in second remission  Chronic or accelerated phase CML Severe aplastic anemia  <b>Selected patients</b>  MDS Lymphoma Myeloma CLL Thalassemia, Sickle cell anemia Severe inherited metabolic diseases	<b>Selected patients</b>  Myeloma Lymphoma Acute leukemia Autoimmune disease e.g. Scleroderma

## **Disease That Have Been Treated Using Cord Blood**

### **1. Malignancies:**

- Acute lymphocytic leukemia (ALL)
- Acute myelogenous leukemia (AML)
- Chronic myelocytic leukemia (CML)
- Myelodysplastic syndrome (MDS)
- Solid tumors: Neuroblastoma, Non-Hodgkin's lymphoma

### **2. Inborn Errors of Metabolism**

### **3. Hemoglobinopathies & Blood Disorder**

- Amegakarocytic thrombocytopenia (AMT)
- Aplastic anemia and Fanconi's anemia
- Blackfan-Diamond anemia
- Congenital cytopenia
- Evan syndrome
- Kostmann syndrome
- Sick cell anemia
- Thalassemia

### **4. Immunodeficiencies**

- Adenosine deaminase deficiency (ADA or SCID-ADA)
- Severe combined immunodeficiency diseases (SCIDs)
- Wiskott-Aldrich syndrome
- X-linked lymphoproliferative disease (XLP)

### **5. Malignant Diseases**

- Juvenile chronic myelogenous leukemia
- Neuroblastoma
- Refractory anemia with excess blasts

### **6. Non-Malignant Diseases**

- Refractory anemia and globoid cell leukodystrophy

# APPENDIX 1

## HEMATOLOGICAL TESTS AND NORMAL VALUES

TEST	NORMAL VALUE	MAIN PURPOSES	METHOD
Hemoglobin (Hb)	15.5 +/- 2.5 gm/dL (Male) 14 +/- 2.5 gm/dL (Female)	Testing for low and high blood cell counts; anemia, polycythemia	Automated particle counting instruments aspirate, dilute and analyze one blood specimen in about a minute, impedance, laser, and /or radiofrequency-based methods are used for cell counts. Backup manual methods include "hemoglobinometer" spectrophotometry of cyanomethemoglobin, hematocrit centrifugation, hemocytometer chamber cell count, blood smear estimate of WBCs.
Hematocrit (Hct)	47 +/- 7% (Male) 45 +/- 5% (Female) 38 +/- 4% (Child)		
Red Blood Cell Count (RBC)	5.5 +/- 1x10 <sup>6</sup> /μL (Male) 4.8 +/- 1x10 <sup>6</sup> /μL (Female) 4.7 +/- 0.7x10 <sup>6</sup> /μL (Infant/Child)		
Red Cell Distribution Width (RDW)	11.5-14.5%	Detection of anisocytosis, or variation of red cell size.	
RBC Mean Cell Volume (MCV)	82-102 fL (Male) 78-101 fL (Female)	One form of initial categorization of type of anemia, i.e macrocytic, normocytic, microcytic	
White Blood Cell Count (WBC)	4.1-10.9x10 <sup>3</sup> /μL (details page 15)	Leukopenia causes Leukocytosis causes	
Differential:	Polymorphonuclear Cells (polys) 35-80% Immature Polys (bands) 0-10% lymphocytes (lymp) 20-50% monocytes (mono) 2-12% eosinophils (eos) 0-7% basophils (bas) 0-2%	Looking for patterns of infection, bacterial or viral; allergy, or leukemia	A drop of blood is smeared on a glass and stained with Wright's stain. White cells seen are tallied into categories while the smear is scanned under a microscope. Meanwhile, WBC and platelet count can be estimated, red cell morphology reviewed.
Platelet Count (Plt)	140000-400000/μL	To look for causes of bruising or bleeding, which is common with very low counts	1. Automated 2. Chamber count 3. Blood smear



Reticulocyte	0.5-1.5% (Adult) 1.1-4.5% (Newborn) 0.5-3.1% (Infant)	Determining categories of anemia (e.g. hemolysis and response of anemia therapy)	Automated and manual methods
Erythrocyte sedimentation rate	<20 mm/h	Screening for illness (e.g. inflammatory)	Blood cells are allowed to sediment for one hour in a calibrated tube.
Prothrombin Time (PT)	12-14 seconds	See coagulation	
Partial Thromboplastin Time (PTT)	18-28 seconds	See coagulation	
Fibrinogen	170-420 mg/dL Higher in normal pregnancy and in inflammatory states.	Screening for DIC, checking validity of specimen if PT/aPTT prolonged in patient with an indwelling line.	
Bleeding Time , Ivy, simplate	< 9 minutes	Screening test for the detection of platelet dysfunction	
Serum iron	Iron 50-160 µg/dL	Detection of iron deficiency (low iron, high IBC, low% sat) or iron overload ( high iron, high % sat)	Automated chemistry instruments; detected by color change after combining with chromogen ferrozime
Iron-Binding Capacity (TIBC)	IBC 250-470 µg/dL		
Transferrin	204-360 mg/Dl		
Ferritin	18-250 ng/mL (Male) 12-160 ng/mL (Female)	Detection of iron deficiency and iron overload. The test is less liable than serum iron.	Radioimmunoassay; alternatively the IMX instrument sandwich technique with an Elisa endpoint.
Plasma or serum free hemoglobin	<4 mg/dL	Detection of intravascular hemolysis	Patient's plasma or serum mixed with a chemical that generates color in the presence of the peroxides activity of hemoglobin
Hemosiderin in urine	None seen in centrifuged first AM or any other urine	Detection of recent, usually persistent, intravascular hemolysis	Urine sediment is stained by Prussian Blue technique and examined microscopically for iron particles
Pyruvate kinase screen	Normal activity disappearance of florescence	Detection of congenital deficiency of PK enzyme that causes hemolytic anemia.	Patient's hemolysate is mixed with chemicals that will destroy NADPH fluorescence if PK is present.
Erythrocyte protoporphyrin	10-28 µg/dL whole blood	Screens for lead poisoning and iron	

		deficiency	
Hb-electrophoresis Alkaline (pH 8.6) supporting media include cellulose acetate, agarose, or acrylamide, locally	Hemoglobin A, F and A2 in that order, are usually seen as separate bands	Detection of hemoglobinopathies able to cause anemia, hemolysis, sickling complications, cyanosis, polycythemia	Red cells are washed with saline. then lysed with water+/- toluene; hemoglobin is separated from RBC membrane by centrifugation then applied to the buffer saturated supporting medium and subjected to an electric current for 20 min or more. Hbs move toward the positive pole at alkaline pH. Bands are quantitated by densitometer.
Hb-electrophoresis, acid (pH 6.2) - agarose	Hemoglobins F, A, and A2 are seen	Further definition of abnormal hemoglobins seen at pH 8.6. Discriminates Hb S from D and G; C from E and O arab. Discriminates well between AS and SS in newborn	Hemolysis prepared as above is applied to an agarose gel and subjected to an electric current. Hbs move toward negative pole.
Hb A2 quantitation by minicolumn	< 3.8% of total Hb	By identifying patients with elevated Hb A2 one detects $\beta$ -thalassemia trait.	Prepared column is allowed to settle in upright position. Hb applied to top; buffers poured through; Hb fractions collected; Hb levels read using Drabkin's solution, % Hb A2 calculated.
Sickle cell preparation	No sickling	Detection of sicklable red cells (Hb S; rarely Hb C-Harlem).	Red cells are mixed with a strong reducing agent, sodium metabisulfate, and watched, off and on, for 1 hour sealed under a cover slip for characteristic shape change.
Fetal hemoglobin	< 2% after the age of 6 months	Detection of thalassemias major and minor, including beta-delta thalassemia and hereditary persistence of fetal Hb. Monitoring of patients with sickle cell anemia treated	Dilute, KCN treated hemolysate is mixed with 1/15 vol of 1.2 N sodium hydroxide, which denatures nonfatal hemoglobins, precipitated with ammonium sulphate, then poured through

		with sickle cell anemia treated with hydroxyurea (Hb F may increase to 25%)	a filter. The amount of hemoglobin filtered is compared with hemoglobin in the starting material.
Fetal red cell stain: Kleihauer-Betke	No Heinz bodies	Detection of fetal red cells in maternal blood; identification of red cells in the amniotic fluid; study of distribution of fetal Hb among red cells. Used for dosing Rhogam after Rh incompatible delivery and for diagnosis of hereditary persistence of fetal hemoglobin (HPFH)	Blood smear is prepared; adult Hb is eluted selectively using citrate buffer. Residual Hb is stained. In Rh incompatibility, fetal Hb containing RBCs are counted. In HPFH diagnosis distribution of fetal Hb among RBCs is elevated.
Heinz body, supravital RBCs stain	No Heinz bodies	Detection of unstable Hbs (e.g. Hb Köln) or mechanism of oxidant mediated hemolysis by discovery of denatured Hb bodies in RBCs	Red cells are mixed with crystal violet stain in a test tube, incubated, and then inspected for Heinz bodies. Positive controls are made treating normal RBCs with acetyl phenyl hydrazine.
Hb H inclusion, supravital stain No inclusions	No inclusions	Confirmation at Hb H (or Hb barts) inclusions	Red cells are mixed with crystal blue stain, incubated, smeared, inspected for inclusions.
Thrombin Time	Adjusted like thrombin time: 20 sec	Detection of heparin effect, or abnormal fibrinogen, or fibrin split products; lytic state in fibrinolytic treatment. Can be used for heparin anticoagulation control.	Clotting time of patient's plasma, straight or diluted, after addition of dilute bovine thrombin.
Fibrin degradation or split products (FDP or FSP), Fragments D and E	<10µg/mL	Screening for DIC, fibrinolysis	Serum obtained from blood drawn and clotted in special tube is mixed with latex particles coated with antibody to fibrin or fibrinogen. Particles are then observed for clumping.
D-dimer	<0.5µg/mL	Screening for DIC;	Latex beads coated



		also may be useful in detection of venous thrombosis	with anti D-dimer are clumped on a glass plate if D-dimer is present in patient plasma.
Coagulation factor assays (II, V, VI, VIII, IX, X, XI, XII)	50-150 % (most) 50-200% (VIII)	Testing for congenital and acquired coagulation disorders	Clotting, detected by time to turbidity changes, of factor deficient plasma; Substrate is corrected, by patient's plasma as the source of the factor being tested.
Platelet aggregation ( with collagen, ADP, epinephrine, Ristocetin, arachidonic acid)	>50% clearing of patient's PRP after addition of each agent	Detection and partial characterization of platelet dysfunction.	Patient's blood is gently centrifuged to prepare platelet-rich plasma (PRP); aggregating agents are added to aliquots of PRP, and PRP is monitored for aggregation.
Antithrombin III (Natural anticoagulant that inhibits thrombin, factor Xa, others; it is activated by heparin.	70-130%	Detection of AT III deficiency or dysfunction	Patient's plasma is used for anti factor Xa effect after mixing plasma with Xa, heparin, chromogenic substrate
Protein C ( natural anticoagulant that when activated, degrades activated clotting f V and VIII)	70-130%	Detection of protein C deficiency or dysfunction	Patient's protein C is activated and in turn converts a chromogenic substrate
Protein S	70-130%	Detection of deficiency of free protein S	Laurell rocket antigen precipitation before and after an initial REG precipitation of bound PS.
Bone marrow aspiration (combined with marrow biopsy, flow cytometry, special cytochemical stains, chromosome analysis, and cultures.	Erythroid series 22% Myeloid (N, E, B) 58. Lymphocytes 16% Monocytes 2% Plasma 2% M/E ratio 2.5:1	Looking for causes of abnormal blood counts, benign and malignant. Occasionally, staging malignancies despite normal counts; obtaining specimen for cultures	Marrow is aspirated into syringe containing 1% EDTA, smeared, stained with Wright-Giemsa and Prussian B. stains and reviewed by hematologist.
Flow cytometry	Normal and abnormal blood cells have characteristic arrays of surface	Detection and identification of leukemia and lymphoma, including subtyping	Mononuclear cells collected from PB or BM by Ficoll-Hypaque separation or electronically, after

	antigens by which they can be identified, tallied, and separated if necessary.	of these conditions. Detection of residual disease after treatment. Assessment of marrow recovery after transplantation. Tallying of CD 4 and CD8 cells in HIV disease and CD34 cells in stem cell collections for marrow transplantation.	lysing red cells. They are labelled with fluorescent-tagged monoclonal antibodies (MABs) and passed through a laser beam. Light is scattered to fluorescence detectors and cells with surface antigens are tallied
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## APPENDIX II

### CD NOMENCLATURE FOR LEUKOCYTE ANTIGEN

Cell surface markers are molecules in the cell membrane that can be recognized by reactivity with specific monoclonal antibodies. Their presence gives information about the lineage, function or stage of development of a particular cell population. The cluster of differentiation (CD) nomenclature system groups together antibodies recognizing the same surface molecule.

Antibody (CD)	Reactivity
CD1a	Thymocytes and immature T cells
CD2	T cells, Large granular lymphocytes ( <u>LGL</u> ), NK cells, some <u>APL</u> , neoplastic mast cells
CD3	T cells, primary effusion lymphoma
CD4	T cells (helper/inducer), monocytes, myeloblasts, NK cell lymphoma
CD5	T cells, <u>B-CLL/SLL</u> , <u>MCL</u>
CD7	T cells, some myeloblasts
CD8	T cells (suppressor/cytotoxic), large granular lymphocytes ( <u>LGL</u> )
CD10	Follicle center cells, <u>FL</u> , some <u>DLBCL</u> , <u>pre-B-ALL</u> , <u>pre-T-ALL</u> , thymocytes, <u>BL</u>
CD11b	Granulocytes, monocytes
CD11c	Monocytes, <u>HCL</u> , <u>LGL</u> , activated T cells, <u>MZL</u>
CD13	Myeloid cells, rare <u>pre-B-ALL</u>
CD14	Monocytes
CD15	Granulocytes, Hodgkin's lymphoma
CD16	Granulocytes, NK cells, <u>LGL</u>
CD19	B cells, <u>pre B-ALL</u> , subset of <u>AML</u> ( <u>AML1/ETO</u> with

	t(8;21))
CD20	B cells, rare plasma cell myelomas
CD22	B cells
CD23	<u>B-CLL/SLL</u> , plasma cells, follicular dendritic cells
CD25	<u>HCL</u> , subset of B and T cell lymphomas
CD30	Hodgkin's lymphoma, anaplastic large cell lymphoma, subset of <u>DLBCL</u> , subset of B cell lymphoma
CD33	Myeloid cells, rare <u>pre B-ALL</u> , rare blastic NK lymphoma
CD34	myeloblasts, lymphoblasts, endothelial cells
CD38	Plasma cells, activated T and B cells, subset <u>B-CLL/SLL</u> , epithelial cells
CD41	Megakaryocytes
CD43	Myeloid cells, T-cell lymphoma, <u>pre B ALL</u> , <u>pre T ALL</u> , B cell lymphoma (subset), plasma cells
CD56	NK cells, <u>LGL</u>
CD57	NK cells, <u>LGL</u>
CD61	Megakaryocytes
CD79a	B cells, plasma cells, megakaryocytes
CD103	<u>HCL</u> , rare T cell lymphomas
CD117	<u>AML</u> , mast cells, stromal tumors (GIST), plasma cells
bcl-2	Mature B cells, (except benign GCC), T cells, and <u>FL</u>
Heavy Chains (IgG, IgA, IgM, IgD)	B cells, plasma cells, <u>DLBCL</u> with ALK expression
HLA Dr	<u>AML</u> (except <u>APL</u> ), B cells monocytes
Light chains (kappa or lambda)	B cells (surface), plasma cells (cytoplasmic)
TdT	<u>pre-B-ALL</u> , <u>pre-T-ALL</u> , some <u>AML</u> and hematogones



### APPENDIX III CHEMOTHERAPEUTIC AGENTS AND BIOLOGICS

DRUGS (TYPE OF DRUGS)	Administration	MECHANISM	INDICATION	SIDE EFFECTS
Amsacrine (m-AMSA) (Alkylating agent)	Slow IV infusion  PO (investigational)	Alkalates and intercalates DNA, inhibitors of topoisomerase II	AML., ALL	Severe myelosuppression, cardiac toxicity, nausea, vomiting, mucositis
Asparaginase (Enzyme)	IM Slow IV push (over 30 minutes)	Deplete cells of L-asparagine	ALL	Hypersensitivity, anaphylactic reaction, fever, bronchospasm hyperglycemia, increase liver enzymes
Bleomycin (Antibiotic)	IM, IV, SC Intraarterial Intratumoral Intracavitary	Intercalates DNA, induces DNA breaks (acting at G2 and M phases)	Lymphoma, malignant tumors	Fever, myalgia, anorexia, skin pigmentation, rash, mucositis, alopecia, pneumonitis
Carbaplatin (Alkylating agent)	Brief IV infusions (over 15 minutes); continuous 24-h IV infusion; intraperitoneal	Produces DNA- DNA interstrand cross-links. Produces DNA- protein cross links	Bladder, Ovarian Endometrial Head & neck Non-small cell lung Testicular	Anemia, Diarrhea, Nausea, Hematuria, Hepatotoxicity, Leukopenia Thrombocytopenia
Carmustin (BCNU) (Nitrosurea)	Slow IV infusion (1-2 h)	Bifunctional alkylating agent, induces DNA breaks	Lymphoma, solid tumors	Myelosuppression, lung toxicity, nausea, vomiting, in some cases hepatic and renal toxicity
Chlorambucil (Alkylating agent)	PO	Alkylates DNA, RNA, induces DNA breaks	CLL, other low grade lymphoma	Leukopenia, GI discomfort,
Cladribine (2- CDA) (Alkylating agent)	Continuous IV infusion	Alkylates DNA, RNA, induces DNA	CLL, other low grade lymphoma	Leukopenia, GI discomfort
Cyclophosphamide  (Bifunctional alkylating agent)	PO IV	Alkylates DNA and RNA, induces DNA breaks	Lymphoma, leukemia, solid tumors, immunosup- pression, conditioning for bone marrow transplantation	Myelosuppression, thrombocytopenia, nausea, vomiting, mucositis, fever, hemorrhagic cystitis, tubular nephropathy
Cytosine- Arabinoside (ARA-C) (Antimetabolite)	SC, IM, IV push, Continuous IV infusion, intraperitoneal	Incorporate into DNA, inhibits DNA polymerase, S- phase specific	Acute and chronic leukemias, malignant lymphoma	Myelosuppression, GI toxicity, pulmonary toxicity, alopecia, rash neurotoxicity,
Dacarbazine	IV push, IV	Methylate DNA	Lymphoma,	Nausea, vomiting,

(DTIC) (Alkylating agent)	infusion, Intraarterial , IT, Intraventricular		other malignancies	myelosuppression, alopecia
Daunorubicin			Acute leukemia	See Doxorubicin
Doxorubicin (Anthracycline antibiotic)	IV push, cont. IV push, Intraarterial, topical bladder instillation	Intercalates DNA, forms free oxygen radicals, inhibits topoisomerase II	Lymphoma, solid tumors	Myelosuppression, acute and chronic cardiotoxicity, mucositis, nausea, alopecia
Epirubicin			Lymphoma	See Doxorubicin
Etoposide (VP-16) (Derivative of epipodophyllotoxin "alkaloid")	PO, IV infusion, Intrapleural, Intraperitoneal	Complexes with topoisomerase II, induces DNA breaks	Leukemia, lymphoma, Solid tumors	Bone marrow depression, alopecia, allergy
Fludarabine (Antimetabolite)	Short IV infusion Rapid loading dose/continuous IV infusion	Inhibits enzymes of DNA synthesis	Inhibits enzymes of DNA synthesis	Low grade lymphoma
Hydroxyurea (Antimetabolite)	PO	Inhibits ribonucleotide reductase	CML, other myeloproliferative disorders	Myelosuppression, GI toxicity,
Idarubicin	IV brief infusion (10-15 min).		Acute leukemia , lymphoma	See doxorubicin
Ifosfamide (Alkylating agent)	IV	Alkylates DNA and RNA, induces DNA breaks	Lymphoma, other malignancies	Myelosuppression, GI side effects, hemorrhagic cystitis, CNS toxicity
Lomustine (CCNU) (Alkylating agent)	PO	Alkylates DNA and RNA,	Hodgkin's disease, Multiple myeloma, solid tumors	Nausea , stomatitis, alopecia,
Melphalan (Alkylating agent)	PO, Slow IV infusion, Intraarterial, Intraperitoneal	Cross-links DNA	Multiple myeloma, solid tumors	Nausea, protracted myelosuppression
6-Mercaptopurine (Antimetabolite)	PO, IV push, cont. IV push	Inhibits purine synthesis	Acute leukemia	Nausea, Bone marrow toxicity, liver toxicity
Methotrexate (Antimetabolite)	PO, IM, IV, Intraarterial, IT	Inhibits dihydrofolate reductase	Leukemia, lymphoma, other malignancies	Myelosuppression, severe mucositis, nausea diarrhea, hepatic, renal and pulmonary toxicity
Procarbazine (Alkylating agent)	PO, IV	Alkylates DNA, methylates nucleic acid	Lymphoma NHL, Myeloma	Myelosuppression, skin toxicity, nausea, vomiting
6-Thioguanine (Antimetabolite)	PO, IV, Intraperitoneal	Inhibit purine synthesis	Leukemias	Myelosuppression, Nausea, diarrhea

Vincristine, Vindesine (Vincalcaloids)	Slow IV push, continuous IV push	Inhibit function of microtubuli, inhibit DNA- dependent RNA polymerase	Lymphoma, leukemia, other malignancies	Nausea & vomiting, diarrhea, pulmonary toxicity, mouth ulcer, myelosuppression
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## APPENDIX IV

### DRUGS USED IN HEMATOLOGY

Drug	Mechanism	Indication	Common side effects
<b>A. ANTICOAGULANT</b>			
Heparin	1. A catalyst to antithrombin III 2. Prolonged PT and PTT	1. MI 2. DVT 3. Stroke, acute	1. Bleeding leading to stroke 2. Heparin induced thrombocytopenia 3. Prolonged use: osteoporosis
Low molecular weight heparin	1. Activates antithrombin III	1. Thrombosis prophylaxis 2. Non-hemorrhagic stroke 3. Hemodialysis	1. Bleeding 2. Thrombocytopenia is rare
Warfarin	1. Inhibits vitamin K dependent clotting factor II, VII, IX and X from undergoing gamma carboxylation in liver 2. Prolonged PT or INR	1. DVT 2. PE 3. Atrial fibrillation 4. 2-6 month after MI	1. Bleeding and hemorrhagic stroke 2. Teratogenic 3. Cutaneous necrosis during 1 <sup>st</sup> week of therapy
<b>B. DRUGS FOR ANEMIA</b>			
Iron	For synthesis of hemoglobin	1. Iron deficiency anemia treatment and prevention 2. Pregnancy	In children: acute toxicity as necrotizing enterocolitis, shock, metabolic acidosis, coma and death.
B12	Synthesis of folic acid and DNA	B12 deficiency	No significant toxicity
Folic acid	Synthesis of	1. Folic acid	No significant



	purines and thymidylate thus DNA	deficiency 2.Pregnancy	toxicity
Erythropoietin	Stimulate RBC synthesis	1.Renal failure 2. Marrow failure 3. Myelodysplastic syndrome 4.Autologous blood donation	No significant toxicity
<b>C. BIOLOGICS</b>	<b>MECHANISM</b>	<b>INDICATION</b>	<b>SIDE EFFECTS</b>
Filgrastin G-CSF, Neupogen SC. IV 5µg/kg/day administered as a single daily dose for up to 2 weeks	1.Promotes proliferation and differentiation of neutrophils 2. Enhances functional properties of mature neutrophils	Decrease the incidence of infection in neutropenic patients	Bone pain
Sargramustin GM-CSF Leukomax 250µg/m <sup>2</sup> /day for 21 days as a 2-4 hour infusion beginning 2-4 hours after autologous BMT	Stimulates granulocyte and monocyte proliferation	Acceleration of myeloid recovery in patients with NHL, ALL, Hodgkin's disease undergoing BMT	Bone pain, Capillary leak syndrome, fever, fluid retention, headache, hypoxia, hypotension, and pericarditis.
Immunoglobulin (Sandoglobulin, Gamastin Immunodeficiency syndrome: 200-400 mg/kg/month  ITP: 400 mg/kg/day for 2-5 days or 1 g/kg/day for 1-2 days	Provides passive immunity against many infection	1.Prevention of bacterial infection in patients with B-CLL 2. Idiopathic thrombocytopenia	Back pain, chest pain, cyanosis, headache, hip pain, malaise, nausea, urticaria, nephritic syndrome
Interferon α-2a INF-A, Intron A, Roferon-A	1. Direct inhibition of tumor cell growth 2.Modulation of host immune response	1.AIDS related kapos's sarcoma 2.CML 3.Hairy cell leukemia 4.Malignant	Alopecia, anemia, anorexia, dry skin, abnormal LFT Flulike syndrome Insomnia Leg cramps

	3.Activation of natural killer cells 4. Induction of major histocompatibility antigens	melanoma 5.Mycosis fungoides 6.NHL 7.Renal cell carcinoma	Myelosuppression Neutropenia
Interferon $\alpha$ -2b HCL: 2 million IU/m <sup>2</sup> , 3 times a week Kapos's: 30 million IU/m <sup>2</sup> 3 times a week	1.Modulates the immune response 2.Has an antiproliferative effect on tumor cells	1.AIDS-associated kapos's sarcoma 2. Chronic hepatitis 3. Hairy cell leukemia	Arthralgia, fever, headache, hypotension, myalgias, phlebitis, pulmonary infiltrate.

## GLOSSARY

**Absolute reticulocyte count- reticulocyte/1.** The reticulocyte count (%) is divided by 100 and multiplied times the RBC count/1.

**Acanthocyte** Speculated erythrocyte with variably spaced and sized, blunted projections.

**Acute lymphocytic leukemia (ALL)** - A rapidly progressing cancer of the blood in which too many immature (not fully formed) lymphocytes, a type of white blood cell, are found in the bone marrow, blood, spleen, liver, and other organs.

**Acute myelogenous leukemia (AML)** - A rapidly progressing cancer of the blood in which too many immature (not fully formed) granulocytes, a type of white blood cell, are found in the bone marrow and blood.

**Adjuvant therapy** - Treatment used in addition to the main treatment. Adjuvant therapy usually refers to hormonal therapy, chemotherapy, radiation therapy, or immunotherapy added after surgery to increase the chances of curing the disease or minimizing symptoms.

**Agglutination-** Clumping of cells together in clusters. It is caused by the presence of surface immunoglobulins on erythrocytes and expression of surface adhesion molecules on leukocytes and platelets.

**Aggregation-** In blood coagulation, the process in which platelets stick or clump together

**Agranulocytes** - The leukocytes which lack specific granules; however, these cells may or may not contain azurophilic granules. Agranulocytes are spherical in shape, contain nuclei and include lymphocytes and monocytes. These cells are part of the formed elements of whole blood. Whole Blood - A general description for a sample of blood taken from the venous or arterial circulation. It is composed of blood cells, platelets, and plasma

**Aleukemic leukemia-** A form of leukemia, in which little change is seen in the total leukocytes count or cellular maturity in the peripheral blood. An increase number of immature cells can be found in the bone marrow.

**Allogeneic bone marrow transplantation** - A procedure in which a person receives stem cells from a compatible donor.

**Anemia** - A blood disorder caused by a deficiency of red blood cells or hemoglobin (the oxygen-carrying protein in red blood cells).



**Anisochromia-** Variation of the color of erythrocytes due to unequal hemoglobin concentration

**Anisocytosis** - Presence of red cells with increased variability (heterogeneous in size) as measured by red cell distribution width (RDW).

**Antihemophilic factor;** Factor VIII

**Anti-human globulin test (AHG)** Previously referred to as the Coomb's test. May be either a direct or an indirect test to detect the presence of antibodies on erythrocytes "indirect test"

**Antithrombin III-** An alpha-2globulin, that circulates in the plasma.

**Apo ferritin-** A protein, that combines with iron to form ferritin.

**APTT-**Activated partial thromboplastin time

**Apheresis** - A procedure in which a patient's own blood is removed, particular fluid and cellular elements are extracted from the blood, and then returned to the patient.

**Aplasia-** Few or no differentiated cells present (e.g., erythroid aplasia indicates a lack of erythroid precursors in bone marrow.)

**Aplastic anemia** - One type of anemia that occurs when the bone marrow produces too few of all three types of blood cells: red cells, white cells, and platelets.

**Auer rods or Auer bodies-**These cellular inclusions are aggregates of cytoplasmic granules that appear as red, elongated structures. They may occur alone or in groups in myeloblastic and occasionally in monoblastic.

**Autologous bone marrow transplantation** - A procedure in which a patient's own bone marrow is removed, treated with anticancer drugs or radiation, and then returned to the patient.

**Autosomal dominant-**A genetic trait that expresses itself, if present, and is carried on one of the chromosome pairs 1 through 22

**Autosomal recessive inheritance** - A gene on one of the first 22 pairs of chromosomes, which, when present in two copies, causes a trait or disease to be expressed.

**Azures** - A type of dye found in Romanowsky-type stains. Azure dyes are oxidation products of methylene blue and stain certain components of a blood smear reddish blue in color. Blood smear components which have an affinity for this stain are said to display azurophilia. Thus, some cells may contain azurophilic granules.

**Azuophilic Granules** - Cytoplasmic granules which may be present in all types of leukocytes as seen using Romanowsky-type stains. These are usually reddish blue in color, but vary in color intensity, number, and size within different leukocytes. These granules may be absent in certain forms of lymphocytes and monocytes, but not in normal granulocytes. Most of the azuophilic granules are lysosomal in nature, but not those of basophils. The affinity of the granules for azure dyes depends on the chemical nature of the granules, not in the functional classification

**Barium swallow/upper GI series** - A diagnostic test that examines the organs of the upper part of the digestive system: the esophagus, stomach, and duodenum (the first section of the small intestine). Fluid called barium (a metallic, chemical, chalky, liquid used to coat the inside of organs so that they will show up on an x-ray) is swallowed. X-rays are then taken to evaluate the digestive organs.

**Basophilic stippling**- Erythrocytes containing blue-staining punctuate inclusions when stained with routine Romanowsky-type blood stain.

**Bence Jones Protein**; The abnormal protein frequently found in the urine of patients with multiple myeloma (50-60% of patients). It precipitates at 50°C, disappears at 100°C, and reappears on cooling to room temperature.

**Bernard-Soulier syndrome**- A disorder characterized by the largest platelets which seen in a patient disorder.

**Blasts** - Immature blood cells.

**Blood** - The life-maintaining fluid which is made up of plasma, red blood cells (erythrocytes), white blood cells (leukocytes), and platelets; blood circulates through the body's heart, arteries, veins, and capillaries; it carries away waste matter and carbon dioxide, and brings nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the tissues.

**Blood banking** - The process that takes place in the laboratory to ensure that the donated blood or blood products are safe, before they are used in blood transfusions and other medical procedures. Blood banking includes typing and cross matching the blood for transfusion and testing for infectious diseases.

**Blood Cells** - The red (erythrocytes) and white (leukocytes) blood cells comprise the minor portion of whole blood.

**Blood Smear** - A laboratory procedure for examination of a small drop of blood spread over a glass slide.

**Bone marrow** - The soft, spongy tissue found inside bones. It is the site of development and storage of about 95 percent of the body's blood cells.

**Bone marrow aspiration and biopsy** - The marrow may be removed by aspiration or a needle biopsy under local anesthesia. In aspiration biopsy, a fluid specimen is removed from the bone marrow. In a needle biopsy, marrow cells (not fluid) are removed. These methods are often used together.

**Bone marrow transplant (BMT)** - The transfusion of healthy bone marrow cells into a person after their own unhealthy bone marrow has been eliminated.

**Buffy Coat** - A thin grayish white layer of white blood cells (leukocytes) and platelets covering the top of the packed red blood cells of a hematocrit.

**Cabot's Rings** - Circular or ring-shaped or figure eight, nuclear fragments found in newly differentiated erythrocytes

**Capillary microscopy** - The capillaries at the base of the fingernails may be examined under the microscope, using an Anglepoise lamp and a drop of immersion oil on the nail bed. Vascular abnormality such as increased capillary tortosity may be demonstrated, usually associated with a prolonged bleeding time.

**Ceruloplasmin**- Copper containing protein necessary for iron transport into the circulation.

**CFU-E**- Colony-forming-units-erythroid

**Chèdiak-Higashi anomaly**- A rare inherited autosomal recessive trait that is characterized by the presence of large granules and inclusion bodies in the cytoplasm of leukocytes. The leukocytic neutrophils display impaired chemotaxis and delayed killing of ingested bacteria

**Chemotaxis**- The release of substances that attract phagocytic cells as the result of traumatic or microbial damage.

**Chromosome**- A chromosome is a grouping of coiled strands of DNA, containing many genes. Most multicellular organisms have several chromosomes, which together comprise the. Sexually reproducing organisms have two copies of each chromosome, one from the each parent.

**Chronic myelogenous leukemia (CML)** - A slowly progressing cancer of the blood in which too many white blood cells are produced in the bone marrow.

**Clotting** - The sealing of a blood vessel with coagulated blood.



**Clot retraction-** when blood has clotted the clot retracts, so that after 1 hour at 37°C normally 42-62% of the original blood volume is serum. If platelets or fibrinogen are deficient it may fail to retract normally and show increased friability.

**Cold agglutination-** antibodies in the plasma that react best at 0°C to 20°C

**Coomb's test-** Agglutination test for the presence of anti-erythrocyte antibodies.

**Complete blood count (CBC)** – It is a measurement of size, number, and maturity of the different blood cells in a specific volume of blood.

**Cooley's anemia-** Thalassemia major is usually equivalent to beta thalassemia in a homozygous form and is sometimes called Cooley's anemia

**Crenation** - A process by which red blood cells shrink or shrivel, giving a notched appearance to the cells' profiles. **Differential Cell Count** - A light microscopic procedure for identifying and counting the number of certain formed elements in a blood smear.

**cDNA-** Strong, cloned copies of otherwise fragile mRNA - the essential messenger element of the genes in the DNA which help in the coding of proteins.

**DNA** (deoxyribonucleic acid)-Is a double-stranded helix of nucleotide which carries the genetic information of a cell. It encodes the information for the proteins and is able self-replicate.

**Down's syndrome** - DS is condition with several symptoms, including a characteristic body type, mental retardation, increased susceptibility to infections, and various heart and other organ abnormalities. It is a result of an extra copy of chromosome 21. In most cases this is a result of one of the parents' gametes not dividing properly. In some cases, it is a result of one of the parents having a chromosomal abnormality in which chromosome 14 and 21 are merged. This can be detected by looking at the parents' chromosomes.

**Eosin** - A type of dye found in Romanowsky-type stains. Eosin is an acid dye and stains certain components of a blood smear yellow to light red in color. Blood smear components which have an affinity for this stain are said to display eosinophilia (acidophilia).

**Euglobin lysis time (ELT)** – This is a test for measuring the amount of fibrinolysin present. Too much fibrinolysin causes hemorrhagic tendency. Too little causes intravascular coagulation (IVC) and may occur as a result of amniotic embolism complicating delivery. For this test a citrated blood sample is required. In the laboratory, the euglobin fraction of the plasma is precipitated, redissolved and allowed to clot.

The time taken for the clot to undergo lysis is the euglobin lysis time, normally 150-300 minutes. If there is too much fibrinolysin, as can occur with carcinoma of the prostate, this time is reduced, to 30 minutes or less. In IVC it is increased.

**Ferritin-** It is a water-soluble storage form of iron. Serum ferritin concentrations have a positive correlation to total body iron store.

**Fibrindex (fibrinogen index)** - This test gives a quick estimate of the patient's fibrinogen level; 2 ml of blood in a prothrombin bottle is required. The result is reported as the time taken for the patient's plasma to clot after it is added to thrombin. Normally it clots in 5-12 second.; in moderate fibrinogen deficiency the time is 12-30 seconds, and in emergencies and following thoracic operations.

**Folic acid deficiency** -A deficiency in a B vitamin known as folic acid, which can cause megaloblastic anemia.

**Gene** - A segment of DNA that codes for a trait such as blood type or eye color, as well as susceptibility to certain diseases.

**Graft-versus-host disease (GVHD)** - The condition that results when the immune cells of a transplant (usually of bone marrow) from a donor attack the tissues of the person receiving the transplant.

**Glycophorin** - A major transmembrane protein unique to erythrocytes. This protein has carbohydrate moieties which permits classification of the erythrocytes into specific subgroups.

**Granulocytes** - These are leukocytes which have specific granules. The three different types of granulocytes have different types of specific granules. Granulocytes are spherical in shape, contain nuclei and include neutrophils, eosinophils, and basophils. These cells are part of the formed elements of whole blood.

**Haptoglobin;** Plasma protein that binds hemoglobin released into plasma following intravascular hemolysis.

**Heinz bodies-** Incubation of defective cells with a reducing agent e.g acetyl phenylhydrazine, causes more Heinz bodies to develop than in normal blood. It composed of oxidized hemoglobin that has precipitated into large inclusions within erythrocytes. They stain red to pale pink with Romanowsk-type stains and blue with reticulocyte stains.

**Hemarthrosis** - Bleeding into a joint.

**Hematocrit** - Hct %) the percentage of packed red blood cells found in a unit volume of whole blood. This parameter is derived from the measured red cell volume and the red blood cell count

**Hematopoiesis** (Hemopoiesis) - The process of formation, development, and differentiation of the formed elements of whole blood.

**Hematologist** - A physician who specializes in the functions and disorders of the blood.

**Hematology** - The scientific study of blood and blood-forming tissues.

**Hemoglobin** - A type of protein in the red blood cells that carries oxygen to the tissues of the body.

**Hemoglobin S-beta thalassemia** - Having one copy of the gene which causes sickle cell anemia (HbS) and one copy of a mutated gene in the beta-chain of hemoglobin; this blood disorder produces a moderate anemia and some symptoms similar to sickle cell anemia.

**Hemolysis** - A process characterized by the alternations in the red blood cells' integrity resulting in the release of hemoglobin into the surrounding medium in which the cells are suspended.

**Hemolytic anemia** - One type of anemia in which the red blood cells are destroyed prematurely.

**Hemophilia (Also called a coagulation disorder.)** - An inherited bleeding disorder caused by low levels, or absence of, a blood protein that is essential for clotting; hemophilia A is caused by a lack of the blood clotting protein factor VIII; hemophilia B is caused by a deficiency of factor IX.

**Hemorrhagic anemia** - Anemia caused by a sudden loss of a large amount of blood.

**Hemosiderin**- Water insoluble storage form of iron. It consists of denatured ferritin and can be visualized easily by light microscopy.

**HLA:** Human leukocyte antigen system; a series of four gene families (termed A, B, C and D) that code for polymorphic proteins expressed on the surface of most nucleated cells. Individuals inherit from each parent one gene (or set of genes) for each subdivision of the HLA system. If two individuals have identical HLA types, they are said to be histocompatible. Successful tissue transplantation requires a minimum number of HLA differences between donor and recipient tissues.

**Howell-Jolly Bodies** - Spherical or ovoid nuclear fragments found in newly differentiated erythrocytes. Red blood cells with these bodies appear in greater numbers after a splenectomy.

**Hyperchromic red cells**- Red cells with a cell hemoglobin concentration mean >41 g/dL.% and absolute count are available on the RBC Research Screen



**Hyperchromia-** Presence of red cells with abnormally increased cell hemoglobin content.

**Hypochromic red cells-** Red cells with a cell hemoglobin concentration mean <28 g/dL. % and absolute count are available on the RBC Research Screen.

**Hypochromia-** Presence of cells with abnormally low cell hemoglobin content.

**Immune system** - The system composed of lymph fluid, lymph nodes, the lymphatic system, and white blood cells that are responsible for protecting the body against infection and disease.

**Immune thrombocytopenic purpura (ITP)** - A blood disorder characterized by an abnormal decrease in the number of blood platelets, which results in internal bleeding. There are two forms of ITP: acute ITP and chronic ITP.

**Immunosuppression** - A state in which the ability of the body's immune system to respond is decreased. This condition may be present at birth, or it may be caused by certain infections (such as human immunodeficiency virus, or HIV), or by certain cancer therapies, such as cancer cell killing (cytotoxic) drugs, radiation, and bone marrow transplantation.

**Immunotherapy** - Treatments that promote or support the body's immune system response to a disease such as cancer.

**Intravenous gamma globulin (IVGG)** - A protein that contains many antibodies and slows destruction of platelets; used in the treatment of ITP.

**Iron deficiency anemia-**The most common type of anemia. It is the lack of iron in blood

**Jaundice** - Yellowing of the skin, eyes, and oral mucosa.

**Left shift-** Increased number of unsegmented neutrophils, triggered by a decrease in the lobularity index with or without absence of a valley in the nuclear cytogram.

**Leptocyte-**Thin cell with increased membrane to volume ratio that may resemble targets (target cell or codocyte) may be seen in iron deficiency and with some forms of liver disease.

**Leukemia** - A cancer of the blood-forming tissue. Leukemic cells look different than normal cells and do not function properly.

**Leukocytosis** - A condition characterized by an abnormally high total number of circulating leukocytes.

**Leukopenia** - A condition characterized by an abnormally low total number of circulating leukocytes.

**Lymph** - Part of the lymphatic system; a thin, clear fluid that circulates through the lymphatic vessels and carries blood cells that fight infection and disease.

**lymph nodes** - Part of the lymphatic system; bean-shaped organs, found in the underarm, groin, neck, and abdomen, that act as filters for the lymph fluid as it passes through them.

**Lymph vessels** - Part of the lymphatic system; thin tubes that carry lymph fluid throughout the body.

**Lymphangiogram (LAG)** - An imaging study that can detect cancer cells or abnormalities in the lymphatic system and structures. It involves a dye being injected to the lymph system.

**lymphatic system** - Part of the immune system; includes lymph, ducts, organs, lymph vessels, lymphocytes, and lymph nodes, whose function is to produce and carry white blood cells to fight disease and infection.

**Lymphocytes** - Part of the lymphatic system; white blood cells that fight infection and disease.

**Lymphocytic leukemia** - A type of leukemia in which the cancer develops in the lymphocytes (lymphoid cells).

**Macrocytes** - Cells which are abnormally large, especially red blood cells.

**Maturation arrest**- Situation in bone marrow where maturation of a cell line appears to have largely stopped at a given precursor cell type.

**MCV**-Mean cell volume is the average volume of single erythrocytes in femtoliters (fl).

**Megaloblastic (pernicious) anemia** - A rare blood disorder in which the body does not absorb enough vitamin B-12 from the digestive tract, resulting in an inadequate amount of red blood cells (RBCs) produced.

**Mean corpuscular hemoglobin MCH pg**)- This value is derived from the measured hemoglobin and red blood cell count.

**Mean corpuscular hemoglobin concentration (MCHC g/dL)**-This parameter is computed from the measured hemoglobin and the computed hematocrit. A significant discrepancy between this value and

the directly measured cell hemoglobin concentration mean gives rise to a CE flag.

**M:E ratio-** Myeloid:erythroid ratio is determined by dividing the number of nonlymphoid leukocytes and their precursors counted in the marrow by the number of nucleated erythroid precursor counted

**M:H ratio-** The ratio of microcytes (M) to hypochromic (H) RBCs. This parameter is a simple index that may facilitate differentiation between beta-thalassemia trait and iron deficiency.

**MHC-** Major Histocompatibility complex; a series of genes located on chromosome 6 that code for antigens, including the HLA antigen.

**mRNA** (messenger RNA)-Is the mediating template between DNA and proteins. The information from a particular gene is transferred from a strand of DNA by the construction of a complementary strand of RNA through a process known as transcription. Next three nucleotide segments of RNA, called tRNA (transfer RNA), which are attached to specific amino acids, match up with the template strand of mRNA to order the amino acids correctly. These amino acids are then bonded together to form a protein. This process, called translation occurs in the ribosome, which is composed of proteins and the third kind of RNA, rRNA (ribosomal RNA).

**Methylene Blue** - A type of dye found in Romanowsky-type stains. Methylene blue is a basic dye and stains certain components of a blood smear purplish blue to dark purple in color. Blood smear components which have an affinity for this dye are said to display basophilia.

**Microcytes** - Cells which are abnormally small, especially red blood cells.

**Mucositis** - Inflammation of the mouth and gastrointestinal tract.

**Mutation** - A change in a gene.

**Myelodysplastic syndrome-** MDS consists of abnormal marrow proliferation with morphologic abnormalities resulting in one or more cytopenias. It may be a preleukemic condition.

**Myelogenous leukemia** - A type of leukemia in which the cancer develops in the granulocytes or monocytes (myeloid cells).

**Myelophthisis-** Bone marrow space is occupied by abnormal cells, fibrosis or bone, resulting in peripheral cytopenias.



**Myeloproliferative disorders** - Diseases in which the bone marrow produces too many of one of the three types of blood cells: red blood cells, which carry oxygen to all the tissues in the body; white blood cells, which fight infection; and platelets, which make blood clot.

**NRBC-** Nucleated red blood cell is a term used to refer to nucleated erythrocytes present in blood

**Petechia** - Tiny red dots under the skin that are the result of very small bleeds.

**Plasma** - The noncellular liquid component of unclotted whole blood. Plasma is the liquid medium in which the formed elements are suspended and comprises the major portion of whole blood

**Platelet distribution width (PDW %)** - Derived from direct flow cytometric measurement of platelet cell volume presence of platelet anisocytosis

**Platelet pheresis** - A procedure to remove extra platelets from the blood.

**Platelets** - Cytoplasmic fragments of megakaryocytes (bone marrow cells). Platelets contain cytoplasmic granules; however, they lack nuclei and are part of the formed elements of blood. It is a cell found in the blood that are needed to help the blood to clot in order to control bleeding; often used in the treatment of leukemia and other forms of cancer.

**Polycythemia-** Erythrocytosis with increases in Hct, RBC count and Hb concentration.

**Pure red cell aplasia-** Erythroid aplasia with lack of only erythroid precursors in the bone marrow.

**Pluripotent stem cell** - The most primitive, undeveloped blood cell.

**Prothrombin consumption test-** When healthy blood clots most of the prothrombin is used up. In clotting defects much prothrombin may still remain in the serum. This test measures how much prothrombin remains in the serum. This is the prothrombin consumption index (PCI) normally 0-30%, usually below 10%. A raised result indicates a clotting defect requiring further tests to be described.

**Purpura** - The purple color of skin after blood has "leaked" under it, such as in a bruise.

**Red Blood Cells** - Blood cells (erythrocytes) which appear as biconcave disks, lack nuclei and comprise the largest number of cells of the formed elements of whole blood.

**Red blood cells (Also called RBCs or erythrocytes.)** - Blood cells that mainly help transport oxygen to all the tissues, it is increased in the in the body.

**RDW**-Red cell distribution width is calculated as the coefficient of variation of erythrocyte volume. It indicates the degree of variation of cell volume.

**Reticulocyte red blood cell distribution width (RDW<sub>r</sub> %)**-Derived from direct flow cytometric measurement of reticulocyte mean corpuscular volume, it is increased in the presence of reticulocyte anisocytosis.

**Reticulocytes** - Immature erythrocytes which contain fine thread-like strands (network) of RNA (ribonucleic acids). The RNA strands may be demonstrated by supravital staining with methylene blue.

**Right shift**-Frequent hypersegmented neutrophils seen in blood

**Romanowsky-Type Stains** - Blood smear dyes having modified mixtures of eosin and methylene blue. Wright's and Giemsa's stains are examples of these types of blood smear stains.

**Schistocytes**-Are irregular erythrocyte fragments formed by trauma such as occurs in DIC.

**Serum** - The noncellular liquid phase resulting from the clotting of a sample whole blood or plasma. Serum is equivalent to plasma without its clotting elements.

**Sickle cell anemia** - An inherited blood disorder characterized by defective hemoglobin, where there are two copies of an abnormal hemoglobin gene present (HbSS).

**Sickle cell - hemoglobin C disease** - Having one copy of the gene which causes sickle cell anemia (HbS) and one copy of another altered hemoglobin gene (HbC); this blood disorder is similar to sickle cell anemia.

**Sickle cell - hemoglobin E disease** - Having one copy of the gene which causes sickle cell anemia (HbS) and one copy of another altered hemoglobin gene (HbE); this blood disorder may/may not cause symptoms except under stress (exhaustion, infection, etc.).

**Sickle cell trait** - Having one copy of the gene which causes sickle cell anemia (HbS), and one copy of the normal hemoglobin gene.

**Stem cells** - The blood cells that produce other blood cells. It is the stem cells that are needed in bone marrow transplantation.

**Sickle crisis (Also called pain crisis or vasoocclusive crisis.)** - In sickle cell diseases, the pain that occurs when the flow of blood is blocked to an area because the sickled cells are stuck in a blood vessel.

**Spectrin** - The major cytoskeleton protein, along with certain integral proteins, responsible for maintaining the biconcave shape of erythrocytes.

**Spherocytes**-Erythrocytes that appear as spheres. They lack central pallor and have smaller diameter than normal on stained blood film.

**Thalassemia** - An inherited blood disorder in which the chains of the hemoglobin (a type of protein in red blood cells that carries oxygen to the tissues) molecule are abnormal; alpha thalassemia is where a mutation occurs in the alpha chain, while beta thalassemia is where the mutation occurs in the beta chain; signs and symptoms of thalassemias vary from mild (little to no symptoms) to severe (life threatening).

**TIBC** -Total iron binding capacity correlates relatively with transferring concentration. This measures the maximum amount of iron with which the protein can combine. It is increased in iron deficiency anemia.

**Transferrin**-Major iron transport protein in plasma

**Viscometry**- This is the measurement of the viscosity of whole blood or plasma and is performed on sequesternated blood. E.g blood viscosity is increased when there is increase in the PCV.

**Red Cell Mass**- This test measures the total volume of all the circulating red cells. Blood is collected by the laboratory staff. The red cells are tagged with radioactive chromium, washed and reinjected into the patient. After 10-20 minutes blood is again collected. The hematocrit and radioactivity are measured. The red cell mass can then be calculated. Normally this is 30 ml/kg for males and 27 ml/kg for females. It is increased in Polycythemia, sometimes to more than twice the normal figure.

**Von Willebrand disease** - A form of hemophilia caused by an abnormality in the von Willebrand factor, which is necessary for platelets to be able to attach themselves to a vein or artery to form a clot to stop bleeding.

**White blood cells (Also called WBCs or leukocytes)** - Blood cells involved in the destruction of viruses, bacteria, and fungi which cause infection.

**Whole Blood** - A general description for a sample of blood taken from the venous or arterial circulation. It is composed of blood cells, platelets, and plasma



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## BIBLIOGRAPHY

For Further reading

Atul Mehata & Victor Hoffbrand: Hematology at a glance 1<sup>st</sup> edition, 2000

Ayalew Tefferi: Primary Hematology. Human Press. 2001

Barbara A. Brown: Hematology: Principles and Procedures. Sixth edition 1993

Barnard D.L, Mcverry B.A: Clinical Hematology; 1989

Bernard M. Babior: Hematology; A pathophysiological Approach. Third edition 1994

Delamore J.A, Liu Yin J.A: Haematological Aspects of Systemic Disease 1990

Denise M, Harmening: Clinical Hematology and Fundamentals of Hemostasis. Third edition. 1997.

Fred J. Schiffman: Hematologic pathophysiology; 1998

Harold R. Schumacher: Acute Leukemia; Approach to diagnosis; 1990

Hoffbrand A.V & Pettit J.E: Essential Hematology; 4<sup>th</sup> edition, 2000, Blackwell Science.Oxford

Hughes-Jones N.C: Lecture Notes on Hematology. Third edition 1979

Lanzkowski; Pediatric Hematology and Oncology, 1995

Lee G.R., ForsterJ., Lukens J.N, Parakevas F& Rodgers G. (eds) 1998 , *Wintrobe's clinical Hematology* (10<sup>th</sup>). Lippincott.

Mehat A.B (1995) self Assessment Color Review of Hematology, Manson Publishing, London

Mary Louise Turgeon: Clinical Hematology "Theory and Procedures. Third edition, 1999

Penington David, Rush B., and Castaldi P.: G.G deGruchy Clinical Hematology in Medical Practice

Reinhold Munker, Erhard Hiller and Ronald Paquette: Modern Hematology; Biology and Clinical Management. 1<sup>st</sup> edition Human Press. 2000

Robert S. Hillmand Kenneth A. Ault: Hematology in clinical practice: A guide to diagnosis and management. Second edition, 1998

Rodak F. Bernadette: Diagnostic Hematology; 1995

Samuel Gross, Stuart Roath: Hematology; A problem-oriented approach; 1996

Simsons: Hematology: A combined theoretical and technical approach 1989.

Steine-Martin E. Anne, Lotspeich-Steininger Cheryl A., Koopke Jhon A: Clinical Hematology; principles, procedures, correlations 2<sup>nd</sup> edition. Lippincot 1998

Stephen H. Robinson and Paul R. Reich: Hematology; Pathophysiology Basis for Clinical Practice: Third edition 1993

William G. Hocking: Practical Hematology; 1983

Albano F, Mestice A, Pannunzio A, Lanza F, Martino B, Pastore D, et al. The biological characteristics of CD34+ CD2+ adult acute promyelocytic leukemia and the CD34 CD2 hypergranular (M3) and microgranular (M3v) phenotypes. *Haematologica*. 2006;91:311-6.

Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. *Leukemia* 1995;9:1783-6

Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias (FAB cooperative group). *Br J Haematol* 1976;33:451-8.

Benett JM, Catovsky D, Daniel MT et al(1980) The French-American-British (FAB) Cooperative Group. Correspondence; a variant form of hypergranular promyelocytic leukemia (M3). *Br J Haematol* 44: 169-70

Benette JM, CatoSky D, Daniel MT et al(1982) Proposals for the classification of myelodysplastic syndromes. *Br J Haematol* 51; 189-99

Bennett JM, Catovsky D, Daniel M-T, et al. Criteria for the diagnosis of acute leukemia of megakaryocytic lineage (M7): a report of the French-American-British cooperative group. *Ann Intern Med* 1985;103:460-2.

Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML MO). *Br J Haematol* 1991;78:325-9.

Berger R, Flandrin G (1984) Determining the nature of cells studied cytogenetically. *Cancer Surveys* 3:423-38



Berger R, Bernheim A, Daniel MT, et al (1985) Cytogenetic studies on acute myelomonocytic leukemia (M4) with eosinophilia. *Leuk Res* 9: 279-88

Brunangelo Falini: new classification of acute myeloid leukemia and precursor-related neoplasm 2010

Catovsky D, Matutes E, Buccheri V, Shetty V, Hanslip J, Yoshida N, Morilla R. A classification of acute leukaemia for the 1990s. *Ann Hematol.* 1991;62:16-21.

Cheson BD, Cassileth PA, Head DR, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol* 1990;8:813-9.

European Group for the Immunological Characterization of leukemia (EGIL), Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. *Leukemia* 1995;9:1783-6.

First MIC Cooperative Study Group. Morphologic, immunologic, and cytogenetic (MIC) working classification of acute lymphoblastic leukaemias. *Cancer Genet Cytogenetic* 1986;23:189-97.

Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting – Airlie House, Virginia, November 1997. *J Clin Oncol* 1999;17:3835-49.

Hayhoe FG. The classification of acute leukaemia. *Blood Rev* 1988;2:186-93.

Hoelzer D., Gokbuer N: Diagnostik und Therapie der akuten lymphatischen Leukaemia des Erwachsenen. *Onkologie* 8 (7) (2002) 672-85

Jaffe ES, Harris NL, Stein H et al: *World Health Organization Classification of Tumours of haematopoietic and lymphoid tissue* f. Lyon, IARC Press, 2001.

Langerak AW, van Den BR, I.L., Boor PP, van Lochem EG, Hooijkaas H, et al. Molecular and flow cytometric analysis of the Vbeta repertoire for clonality assessment in mature TCRalpha/beta T-cell proliferations. *Blood* 2001; 98: 165-173

Maynadié M, Campos L, Moskovtchenko P, Sabido O, Aho S, Lenormand B, et al. Heterogenous expression of CD15 in acute lymphoblastic leukemia: a study of ten anti-CD15 monoclonal antibodies in 158 patients. *Leuk Lymphoma.* 1997;25:135-43.

McKenna RW, Parkin J, Blomfield CD et al (1982) Acute promyelocytic leukemia: a study of 39 cases with identification of a hyperbasophilic microgranular variant. *Br J Haematol* 50:201-14

Sachdeva MU, Ahluwalia J, Das R, et al (2006) Role of FAB classification of acute leukemias in era of immunophenotyping. Indian J Pathol Microbiol: 2006 Oct;49(4):524-7.

Shibata A, Bennett JM, Castoldi GL et al (1985) Recommended methods for cytological procedures in haematology. Clin Lab Haematol 7:55-74

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